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U. S. DEPARTMENT OF AGRICULTURE.
BUREAU OF PLANT INDUSTRY—BULLETIN NO. 243.
B. T. GALLOWAY, *Chief of Bureau.*

HETEROZYGOSIS IN EVOLUTION AND IN PLANT BREEDING.

BY

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[In Cooperation with the Connecticut Agricultural Experiment Station and Harvard University.]

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., January 20, 1912.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 243 of the series of this Bureau a manuscript entitled "Heterozygosis in Evolution and in Plant Breeding," by Dr. E. M. East, Assistant Professor of Experimental Plant Morphology, Harvard University, and Collaborator of this Bureau, and Mr. H. K. Hayes, Plant Breeder of the Connecticut Agricultural Experiment Station. This paper reports results from experiments that have at different times received aid from this Bureau, the Connecticut Agricultural Experiment Station, and the Bussey Institution of Harvard University and should be considered the product of their joint collaboration.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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B. P. I.—724.

HETEROZYGOSIS IN EVOLUTION AND IN PLANT BREEDING.[1]

INTRODUCTION.

When a biologist begins any line of genetic work with either plants or animals he generally has occasion to differentiate his stock into more or less pure types by in-and-in breeding. Frequently in the case of animals, and nearly always in the case of plants that are naturally cross-fertilized, he finds there is a loss of vigor, usually unaccompanied by pathological symptoms. This loss of vigor is generally expressed by a decrease in the size of the individual, but it may be shown by a slight decrease in fertility. The phenomenon, although it probably occurs in all great groups reproducing sexually, is not general, however, for in many animals and in plants that are normally self-fertilized it is unnoticeable.

If after obtaining his "pure" stocks the experimenter has occasion to cross strains that differ in character, he often finds that the reverse phenomenon occurs. The vigor of the hybrid is greater than that of either parent.

These manifestations have been noticed for over a century by plant breeders and for probably two thousand years or more by animal hybridizers. Until the end of the nineteenth century the interpretation of the phenomena, if, indeed, that which is only a paraphrased statement of the facts can be called an interpretation, was that deterioration both morphological and physiological is the direct result of inbreeding, and therefore occasional crossing of genetically distinct blood lines is a necessary requisite to vigor in every sexually propagated species.

Seven years ago an extended series of investigations was started at the Connecticut Agricultural Experiment Station having as their primary object an interpretation of these facts in keeping with the more extended knowledge comprised in modern biology. This paper presents a full account of the views that the writers have come to hold through the data gathered in these experiments, although it has not been thought necessary or advisable to confuse the arguments by overloading it with all of the data in their posses-

[1] Published also as a contribution from the Laboratory of Genetics, Bussey Institution of Harvard University.

sion. It is hoped that an adequate number of facts are cited to support the thesis, and it is sufficient on this occasion to say that not a single fact has been discovered that is irreconcilable with it.

THE PROBLEM.

The experimental data upon which the defense of our thesis is based have been obtained entirely from plants, but observations of animal hybrids and published records lead us to believe that the facts are the same among animals. We believe, therefore, that our conclusions apply alike to the animal and the vegetable kingdoms, for we believe the propositions upon which the arguments are based are applicable to all organisms reproducing sexually. These propositions are:

(1) Mendel's law—that is, the segregation of character factors in the germ cells of hybrids and their chance recombination in sexual fusions—is a general law.

(2) Stimulus to development is greater when certain, or possibly all, characters are in the heterozygous condition than when they are in a homozygous condition.

(3) This stimulus to development is cumulative up to a limiting point and varies directly with the number of heterozygous factors in the organism, although it is recognized that some of the factors may have a more powerful action than others.

We later in this bulletin take up briefly some of the specific reasons for extending these theories to the animal kingdom, but at present we shall confine ourselves to developing the botanical proof.

EARLY INVESTIGATIONS.

The number of cases in which hybridizers have noticed an increase in vigor in crosses between subvarieties, between varieties, and between species is so great that an extended citation of the facts is superfluous. Without exception the horticultural writers of the nineteenth century noted the phenomenon and many of them described it at great length. We have taken some trouble to find out its generality, and have found records of its occurrence in the gymnosperms (Darwin,[1] 1876; Focke, 1881) and pteridophytes (Focke, 1881) as well as throughout the angiosperms. In fact, out of 85 families of angiosperms in which artificial hybrids have been made, instances of hybrid vigor exceeding that of the parent species have been noted in 59.

Kölreuter (1763), the earliest botanist to study artificial plant hybrids—as Darwin notes—gives many exact measurements of his hybrids and speaks with astonishment of their "statura portentosa"

[1] Citations to literature throughout this bulletin refer to the "Bibliography" on pages 49–51.

and "ambitus vastissimus ac altitudo valde conspicua." Later, after having been struck with certain natural adaptations for cross-fertilization, he made a passing remark which plainly showed that he thought nature had intended plants to be cross-fertilized and that benefit resulted therefrom. The hybridists that followed Kölreuter were all interested in the phenomenon, but up to the time of Darwin only Knight and Gärtner attempted to generalize from their observations. Perhaps this was because each one noted the fact that some species hybrids were small and weak. Knight (1799), however, made the somewhat generalized statement that nature had something more in view than self-fertilization and intended that sexual intercourse should take place between neighboring plants of the same species. On the whole, however, Gärtner has given the best expression of the views of the botanical experimenters down to 1849, and for this reason we have translated in full his section on "Wachstum, Luxuriation und Sprossungsvermögen der Bastarde" (Gärtner, 1849, p. 526). He writes as follows:

One of the most conspicuous and common characteristics of plant hybrids is the luxuriance of all their parts, a luxuriance that is shown in the rankness of their growth and a prodigal development of root shoots, branches, leaves, and blossoms that could not be induced in the parent stocks by the most careful cultivation. The hybrids usually reach the full development of their parts only when planted in the open, as Kölreuter (1763) has already remarked; when grown in pots and thus limited in food supply their tendency is toward fruit development and seed production.

Concerning the great vigor of hybrids all observers are agreed; on this point may be cited Kölreuter (1763), Sageret (1826), Sabine Berthollet (1827), W. Herbert (1837), Mauz (1825), and Lecoq (1845). The vigor of a plant can even serve to indicate its hybrid nature in a doubtful case, as Kölreuter has done with *Mirabilis jalapo-dichotoma.*

Besides possessing general vegetative vigor, hybrids are often noticeable for the extraordinary length of their stems. In various hybrids of the genus Verbascum, for example *lychnitis-thapsus*, the stem shoots up 12 to 15 feet high, with a panicle 7 to 9 feet, the six highest side branches 2 to 3 feet, and the stem $1\frac{1}{4}$ inches in diameter at the base; in *Althaea cannabino-officinalis* the stem is 10 to 12 feet; in *Malva mauritano-sylvestris* 9 to 11 feet; in *Digitalis purpureo-ochroleuca* 8 to 10 feet, with panicles 4 to 5 feet; and in *Petunia nyctaginiflоro-phoenicea* and *Lobelia cardinali-syphilitica* 3 to 4 feet each. Prof. Wiegmann also corroborates these observations.

Hybrids in the genera Mirabilis and Datura are especially conspicuous for their enormous size, as Kölreuter has already stated. The different hybrids of *Datura—Stramonio-tatula, quercifolia-ferox, laevi-tatula,* and *laevi-ferox*—grew so large as to be almost treelike, with branches and leaves that nearly weighed down the stems, even before the time for developing their numerous blossoms. Likewise such species hybrids as *Nicotiana suavolenti-macrophylla, Nicotiana rustica-marylandica,* and *Tropaeolum majus-minus* reach a noteworthy height and circumference.

The root system and the power of germination of hybrids are highly correlated with their great vegetative vigor. Many hybrids, therefore, which are not so luxuriant in growth as those just described, for example, Dianthus, Lavatera, Lycium, Lychnis, Lobelia, Geum, and Pentstemon hybrids, put forth stalks easily and therefore are readily propagated by layers, stolons, or cuttings. The observations of Kölreuter

(1763), Sageret (1826), and Wiegmann (1828) agree with ours in this respect. This extraordinary side branching and tillering, as well as the growth of the main stem, in most hybrids continues until late in the fall and in many until frost, as we have observed in *Lobelia syphilitico-cardinalis*, *Petunia nyctaginiflоro-phoenicea*, *Nicotiana suaveolenti-macrophylla*, *Pentstemon gentianoideo-angustifolius*, *Digitalis purpureo-ochroleuca*, *Malva mauritiano-sylvestris*, *Althaea cannabino-officinalis*, etc. Sageret (1826) makes the same statement about *Nicotiana tabaco-undulata*. There are other hybrids, however, that are without this ability to form sprouts, such as *Matthiola annuo-glabra* and those between several Nicotiana species.

Luxuriation expresses itself at times as proliferation; for instance, in *Lychnis diurno-flos cuculi* the receptaculum is changed to a bud that puts forth branches and leaves. If, moreover, the vigor of the hybrids especially affects the stem and the branches, particularly their length, nevertheless the leaves take part in it by becoming larger. Hybrids in the genera Datura, Nicotiana, Tropaeolum, Verbascum, and Pentstemon are examples.

Kölreuter (1763) expresses the opinion that the strength and luxuriance of hybrids continued long after blooming rests upon the fact that the plants are not exhausted and worn out by the production of seed. Similarly, Edw. Blyth (1837) sees in the impotence or sterility of animal hybrids the explanation of their great muscular development, while the considerable size which these hybrids reach in comparison with their parents may be interpreted in the same manner, since capons are able to make a like growth.

But if we take into consideration that: (1) Such a sex condition may exist in dicœcious plants without resulting in the luxuriance shown by hybrids, then the reason given above may be no adequate explanation of that phenomenon. (2) The luxuriance of the hybrid plants is already present and visible before the development of the flowers, although one may not doubt that the derangement of the sexual activities and of the development of those organs is not without consequences to the inner life of these plants and that there may obtain essential difference between the weakening or the entire suppression of one or the other of the sexual activities of the hybrids and of the normal separation of the sexes. (3) Not all partially fertile and sterile hybrids are gifted with an increased vegetative power, since we have observed several absolutely sterile hybrids with weakened and limited vegetative vigor; for example, *Nicotiana grandiflоro-glutinosa*, *N. glutinosa-quadrivalvis*, *N. rustico-suavolens*, *N. suaveolenti-quadrivalvis*, *Dianthus barbato-deltoides*, *D. caucasico-arenarius*, *Verbascum blattaria-lychnitis*, etc.; at the same time many other hybrids keep the growth relationships of the parent plants unchanged. (4) Among all the hybrids that we have observed, those which show the greatest luxuriance in all their parts are precisely those which show the greatest fertility, for example, *Datura stramonio-tatula*, *Datura quercifolio-ferox*, *Tropaeolum majus-minus*, *Lavatera pseudolbio-thuringiaca*, *Lycium barbaro-afrum*, and *Mirabilis jalapo-dichotoma*. (5) Planting partially fertile hybrids, such as *Nicotiana rustico-paniculata* and *Dianthus barbato-chinensis*, etc., in pots makes the production of fruit and seed easier through limiting the vegetative growth, but a sterile plant is never made fertile by this method. Luxuriance is therefore a peculiar quality of several hybrids, although it is not possessed by all in the same degree.

Although the early hybridizers paid more attention to crosses between distinct species than they did to crosses between races that differed by only a few relatively unimportant characters, there is no question but that they noted a very great number of cases where crosses of the latter character gave plants that were remarkable for their vigor. In fact, we have found no record of intervarietal crosses

where delicate or weak hybrids resulted. On the other hand, species crosses sometimes result in hybrids constitutionally feeble. It is obvious, therefore, that a reasonable interpretation of the facts must include an explanation of each category. This matter must be left until later, however, for the work of the early investigators is cited only to show the prevalence of the phenomena under discussion.

Gärtner's researches were followed by but little systematic study of cross and self fertilization in plants until the time of Darwin, and even Darwin's earlier work was confined to the natural means of plant pollination. This early work, mainly a study of pollination in orchids, was summed up in 1862 by the saying "Nature abhors perpetual self-fertilization," a dictum that has become known as the Knight-Darwin law. This important conclusion gave a great impetus to the study of the means of flower pollination throughout the angiosperms. A huge literature of several thousand titles was built up, from which at times important compilations, such as those of Müller (1873) and Knuth (1898), have been made. Every possible variation in flowering habit was argued into an adaptation for cross-fertilization with an ingenuity and zeal similar to that shown by zoologists in their work upon protective coloration and mimicry, and often with as enthusiastic prodigality of extravagant logic. The earnestness of these observers extended our knowledge of the mechanics of pollination in the angiosperms beyond that of any one phase of general botany, yet in the last half of the nineteenth century Darwin was the only scientist who made a systematic experimental inquiry into the physiological effect of cross-pollination compared with self-pollination. The net result of the work of the other observers was simply to show the widespread occurrence of means by which cross-pollination might take place. This fact may be taken to indicate that cross-fertilization is an advantage to a species, but it certainly does not prove that cross-fertilization is indispensable. The many plants naturally self-fertilized preclude it.

Darwin's later experimental work on this subject was so important, both from the standpoint of completeness and brilliancy of analysis, that it must be considered by itself. For this reason we will disregard chronology and conclude this part of our historical summary with the observations of the greatest hybridizer contemporary with Darwin, W. O. Focke. In Focke's fine work "Die Pflanzen-Mischlinge" he gives a chapter on the properties of hybrids, from which the following extract is taken:

> Crosses between different races and different varieties are distinguished from individuals of the pure type, as a rule, by their vegetative vigor. Hybrids between markedly different species are frequently quite delicate, especially when young, so that the seedlings are difficult to raise. Hybrids between species or between races that

are more nearly related are, as a rule, uncommonly tall and robust, as is shown by their size, rapidity of growth, earliness of flowering, abundance of blossoms, long duration of life, ease of asexual propagation, increased size of individual organs, and similar characters.

To undertake a closer examination of the above propositions, it will be necessary to cite a few examples. The following hybrids are abnormally weak: *Nymphaea alba* when crossed with foreign species, Hibiscus, *Rhododendron rhodora* with other species, *R. sinense* with Eurhododendron, Convolvulus, the polyhybrids of Salix, Crinum, and Narcissus. Moreover, it has often been noticed that other hybrid seedlings are somewhat delicate and are brought to maturity with difficulty. Really dwarf growths have been but seldom observed in hybrids; compare, however, certain hybrids of Nicotiana. (Page 285 above, and especially *N. quadrivalvis* × *tabacum macrophylla*, p. 292.) Giant growths are more frequent; note for example Lycium, Datura, Isoloma, and Mirabilis. In size the hybrids generally surpass both the parental species, or at the least they surpass the average height of the two; compare many hybrids of Nicotiana, Verbascum, and Digitalis. Development often goes on with great rapidity, as Klotzsch has emphasized in his hybrids of Ulmus, Alnus, Quercus, and Pinus. Further, the blossoms of hybrids often appear earlier than do those of the parent species, for example, *Papaver dubium* × *somniferum*, many Dianthus hybrids, *Rhododendron arboreum* × *catawbiense*, Lycium, *Nicotiana rustica* × *paniculata*, Digitalis, Wichura's six-fold Salix hybrids, Gladiolus, *Hippeastrum vittatum* × *reginae*, etc., and especially many hybrids of Verbascum. On the contrary, it must be admitted, there are several hybrids that blossom only after a long growth period or not at all, examples of which may be found in the genera Cereus and Rhododendron. Of earlier ripening of the seed independent of earlier blossoming only one example has come down to me, namely Nuphar. Very frequently, one might say very generally, an extraordinary numerical production of flowers has been observed, for example, Capsella, Helianthemum, Tropaeolum, Passiflora, Begonia, Rhododendron, *Nicotiana* (*rustica* × *paniculata*, *glutinosa* × *tabacum*, and others), Verbascum, Digitalis, many of the Gesneraceæ, Mirabilis, and Cypripedium. The size of the blossoms is often increased in hybrids. By crossing two species with flowers of different size, those of the hybrids very nearly reach (not seldom entirely reach) the size of the larger variety. Examples of hybrids with unusually large blossoms are *Dianthus arenarius* × *superbus*, *Rubus caesius* × *bellardii*, and hybrids of *Rosa gallica*, *Begonia boliviensis*, and *Isoloma tydaeum*.

A great capacity for vegetative propagation is very general in hybrids; among the good examples of such a phenomenon may be mentioned Nymphaea, hybrids of *Rubus caesius*, *Nicotiana suaveolens* × *latissima*, *Linaria striata* × *vulgaris*, and Potamogeton. Great longevity may be mentioned as a characteristic of a few hybrids of Nicotiana and Digitalis, ability to withstand cold is especially noticeable in *Nicotiana suaveolens* × *tabacum latissima*, while *Salix viminalis* × *purpurea* is more sensitive to frost than either of the parent species.

These facts point in part to a certain weakness of constitution which is a peculiarity of the hybrid as a result of its abnormal origin and in part to an extraordinary vegetative vigor. An explanation of the last phenomenon, which has been observed much more frequently than the weakness, has only recently been found. The noteworthy experiments of Knight, Lecoq, and others have been familiar for some time, but only through the painstaking experiments of Charles Darwin has the benefit of a cross between individuals and races of one and the same species been clearly demonstrated. The intensification of vegetative vigor in species hybrids is obviously a corresponding experience which requires no especial explanation on the basis of peculiar conditions in hybrids. It was formerly believed that the decreased sexual fertility of

hybrids was compensated by a greater vegetative luxuriance, a conception the untenability of which, as Gärtner showed, is refuted in the simplest manner by the experience that many of the most fertile crosses (Datura, Mirabilis) are at the same time characterized by the most excessive stature.

THE WORK OF DARWIN.

Through Darwin's work we get a very different insight into the meaning of cross and self fertilization. At the beginning of his work the knowledge on the subject gained from the experiments and observations of the older hybridists might be summed up in one sentence: Crosses between varieties or between species often give hybrids with a greater vegetative vigor than is possessed by either parent. To be sure there was also a belief that ill effects result from inbreeding, but this belief was generally confined to the animal kingdom. At the end of Darwin's brilliant experiments, or, rather, brilliant analyses of simple but great experiments, not a single point of the many ramifications into which the problem may be divided but had been fully covered. Unfortunately Mendel's experiments were unknown, and the master key of the situation was not available to him. Had it been we can not doubt that he would have made good use of it.

Darwin's interest in the subject arose of course from its connection with the problem of evolution. If the offspring from a cross-fertilization has an advantage over the offspring of a self-fertilization in the struggle for existence, one can hardly doubt the power of natural selection in fixing the structures of flowers. And this being granted, it is obvious that in many flowers mechanical devices to procure cross-fertilization would have been developed. Having found this to be the case in several plants, he bent all his energies to interpreting all flower structures in the same manner. As stated before, the fascination of the work thus initiated has brought us a huge literature on the subject, some of the arguments of which are fantastic to say the least. Darwin himself never allowed his conclusions to get ahead of his facts, a trait that his followers did not always copy. He firmly believed that self-fertilization was so injurious that plants dependent upon it must ultimately perish, but he frankly admitted the obstacles which self-fertilized families like Leguminosæ placed in the way of his conclusions. If he had known of the vigorous plants that reproduce apogamously no doubt he would have regarded the obstacles more seriously than he did. Nevertheless one must admit that at that time, considering the importance of placing evolution on an impregnable foundation, Darwin did not overstate his conclusions. He proved conclusively the advantage of cross-fertilization and the numerous means by which it is obtained.

If he did not distinguish between the advantage a process may hold forth and the necessity of that process, it was because he was not in possession of all the facts. One does not criticize Darwin, therefore, if in a careful examination of his data in the light of modern knowledge many facts are found that may reasonably have somewhat different interpretations than those originally given.

The first point we will consider is the benefit arising from cross-fertilization. It must be granted from the data already presented that an increase in vigor generally results when different species or markedly different varieties are crossed. It is also perfectly obvious that many plants are naturally designed for cross-fertilization. It can hardly be argued, however, that specific crosses could have had a widespread value in the course of evolution. It must be shown, therefore, that in plants not widely different in character cross-fertilization shows an advantage over self-fertilization. In Table A ("Cross and Self Fertilisation," p. 240) Darwin's results on this subject are given. To be fair, 15 of these experiments should be discarded, because the number of plants measured in the comparison between those crossed and those selfed is less than five. There are 37 experiments left. Of these, the crossed plants were higher in 24 cases, provided an error of 5 per cent is allowed. In 13 cases, then, cross-fertilization showed no definite advantage.

In Table B, where the weights of entire plants are considered, cross-fertilization showed to advantage in 5 experiments out of 8. From these data it seems logical to argue that cross-fertilization between nearly related plants is often a benefit, yet since types that are self-pollinated in nature—legumes, wheat, tobacco, etc.—are among the most vigorous of living plants, it can not be said to be indispensable. Furthermore, about 25 of our most vigorous species of angiosperms have given up sexual reproduction either partially or entirely and have become apogamous.

Did the simple act of crossing produce these beneficial results? If so, why was the advantage due to cross-fertilization not general and without exception? Darwin himself answered these questions. He says (loc. cit., p. 269):

> A cross between plants that have been self-fertilized during several successive generations and kept all the time under nearly uniform conditions does not benefit the offspring in the least, or only in a very slight degree. Mimulus and the descendants of Ipomoea named Hero offer instances of this rule. Again, plants self-fertilized during several generations profit only to a small extent by a cross with intercrossed plants of the same stock (as in the case of Dianthus) in comparison with the effects of a cross by a fresh stock. Plants of the same stock intercrossed during several generations (as with Petunia) were inferior in a marked manner in fertility to those derived from the corresponding self-fertilized plants crossed by a fresh stock. Lastly, certain plants which are regularly intercrossed by insects in a state of nature and which were artificially crossed in each succeeding generation in the course of my experiments, so

that they can never or most rarely have suffered any evil from self-fertilization (as with Eschscholtzia and Ipomoea), nevertheless profited greatly by a cross with a fresh stock. These several cases taken together show us in the clearest manner that it is not the mere crossing of any two individuals which is beneficial to the offspring. The benefit thus derived depends on the plants which are united differing in some manner, and there can hardly be a doubt that it is in the constitution or nature of the sexual elements. Anyhow, it is certain that the differences are not of an external nature, for two plants which resemble each other as closely as individuals of the same species ever do profit in the plainest manner when intercrossed if their progenitors have been exposed during several generations to different conditions.

In other experiments that Darwin performed it was shown conclusively that crosses between individual flowers borne on the same plant conferred no benefit whatever on the progeny. It is evident, therefore, since plants may differ in nonvisible transmissible characters, that differences in transmissible factors alone account for the benefit produced by crossing and are indispensable to its occurrence. This is clearly shown by the fact that even types naturally self-fertilized, such as the garden pea (*Pisum sativum*), showed a remarkable increase in vigor when entirely different strains were crossed. We may well believe, then, that if Darwin's plants used in his Table A had all been heterozygous at the start they would all have showed a considerable difference in favor of the progeny of those continually cross-fertilized. Furthermore, leaving out of consideration our own beliefs, a study of his own experiments (Ipomoea) shows that if his comparisons had been kept up for a considerable number of generations the cross-fertilized stocks would have become so nearly like the self-fertilized stocks in constitution that the advantage due to cross-fertilization would have been small. But to this point we shall again recur.

Let us now consider whether the known effects of inbreeding and crossbreeding are manifestations of the same phenomenon. In "Animals and Plants Under Domestication" he says (vol. 2, p. 89):

The gain in constitutional vigor derived from an occasional cross between individuals of the same variety but belonging to different families, or between distinct varieties, has not been so largely or so frequently discussed as have the evil effects of too close interbreeding. But the former point is the more important of the two, inasmuch as the evidence is more decisive. The evil results from close interbreeding are difficult to detect, for they accumulate slowly and differ much in degree with different species, whilst the good effects which almost invariably follow a cross are from the first manifest. It should, however, be clearly understood that the advantage of close interbreeding, as far as the retention of character is concerned, is indisputable and often outweighs the evil of a slight loss of constitutional vigor.

It is obvious that Darwin believed in a definite accumulation of evil effects from self-fertilization, but his experiments do not justify this view. He is perfectly correct in saying that the good effects of crossing are immediately evident. This is clear when it is remembered that if two plants differ in several transmissible allelomorphs

the first hybrid generation is heterozygous in all these characters, while future generations as a whole are heterozygous in only part of these characters. Furthermore, one may cross two plants differing but slightly and obtain only a small increase in size; he may then recross with a third plant of widely different nature and obtain a great increase. When one inbreeds, however, he relies on chance combinations to eliminate heterozygosis. This occurs through the action of the laws governing probabilities. Many heterozygous combinations are eliminated at once. This lowers the number of such combinations, and, while the percentage of elimination is the same, the effect of the inbreeding decreases. Complete homozygosis is approached as a variable approaching a limit. It may be illustrated by the old story of the dog decreasing the distance from the hare by half at each jump. The effects of inbreeding, therefore, appear to accumulate, while the effects of crossbreeding are immediately manifest. But is the apparent accumulation of evil effects real? And are the effects evil? Our interpretation is that the effects of inbreeding are not to accumulate ill effects, but to isolate homozygous strains. One does away with a stimulus due to heterozygosis, and one sometimes isolates strains with poor transmissible qualities. But one also isolates good strains; strains that remain good in spite of continued self-fertilization. In other words, the apparent evil effects of self-fertilization decrease directly with the number of generations it is practiced, due to the increase in homozygosis. On the theory entertained by us it should come to an end with complete homozygosis; practically, complete homozygosis is difficult to obtain.

Did such a decrease in deterioration actually occur in Darwin's experiments as they were increased in duration? They did. Darwin himself noted the point. He says ("Cross and Self Fertilisation," p. 55):

> As the plants which were self-fertilized in each succeeding generation necessarily became much more closely interbred in the later than in the earlier generations, it might have been expected that the difference in height between them and the crossed plants would have gone on increasing; but so far was this from being the case that the difference between the two sets of plants in the seventh, eighth, and ninth generations taken together is less than the first and second (and third) taken together.

This statement was made concerning his experiments with *Ipomoea purpurea*, which were continued for 10 generations. The ratio of heights of crossed to heights of selfed plants varied from 100 to 68 in the third generation to 100 to 86 in the fourth generation, but in the ninth generation the ratio was 100 to 79, which is higher than that of the first generation. The tenth generation was indeed low, but it may with all fairness be rejected, for Darwin states that the plants were diseased.

We know, further, that Darwin was not dealing with the same strain at the end of his experiments that he was at the beginning. This change was due, as we now know, to the elimination of Mendelian segregates. The plants in the beginning varied greatly in the color of their flowers. Indeed, they varied during the whole time of experimentation; but the color of the later generations was much more uniform than that of the earlier generations. The selfed generations were so uniform, in fact, that his gardener said "they did not need to be labeled."

In this experiment as well as in those with other species, such as *Mimulus luteus* and *Nicotiana tabacum*, remarkably vigorous self-fertilized types appeared. It may be that new transmissible variations arose, but it is unnecessary to assume it. One may account for every result obtained by Darwin by granting the isolation of homozygous Mendelian segregates, accompanied by loss of the vigor due to heterozygosis through self-fertilization.

RECENT INVESTIGATIONS.

Since the time of Darwin, several writers, whose results will be discussed later, have investigated the effect of inbreeding on animals. Botanists, however, have in general been interested only in the superficial results of inbreeding and crossbreeding and have made no attempts until recently to bring together and to correlate our knowledge regarding them.

In 1905, Shull and the senior writer each started independent investigations concerning the effects of inbreeding in maize, which may be regarded as an ideal cross-fertilized species. To supplement these experiments we have made a large series of crosses with species of the genus Nicotiana which are generally self-fertilized, as well as minor observations on other plants. We will not discuss our previous papers (East, 1907, 1908, 1909, 1910; Hayes and East, 1911) as the present paper gives a résumé of those experiments. Concerning Shull's work (1908, 1909, 1910, 1911), we wish to quote his own conclusions for they are stated very concisely. Furthermore, Shull's data and our own, independently obtained, are corroborative in every detail and therefore have greater weight than either alone. Even the additional conclusions drawn from the data presented in this paper are largely an application of the earlier analysis to the broader problems that are legitimately concerned.

Shull's conclusions up to the year 1910 are summarized by him as follows (Shull, 1910):

(1) The progeny of every self-fertilized corn plant is of inferior size, vigor, and productiveness as compared with the progeny of a normally crossbred plant derived from

the same source. This is true when the chosen parent is above the average condition as well as when below it.

(2) The decrease in size and vigor which accompanies self-fertilization is the greatest in the first generation and becomes less and less in each succeeding generation until a condition is reached in which there is (presumably) no more loss of vigor.

(3) Self-fertilized families from a common origin differ from one another in definite hereditary morphological characters.

(4) Regression of fluctuating characters has been observed to take place away from the common mean or average of the several families instead of toward it.

(5) A cross between sibs (sister and brother) within a self-fertilized family shows little or no improvement over self-fertilization in the same family.

(6) A cross between plants belonging to two self-fertilized families results in a progeny of as great vigor, size, and productiveness as are possessed by families which had never been self-fertilized.

(7) The reciprocal crosses between two distinct self-fertilized families are equal and possess[1] the characters of the original corn with which the experiments were started.

(8) The F_1 generation from a combination of plants belonging to certain self-fertilized families produces a yield superior to that of the original crossbred stock.

(9) The yield and quality of the crop produced are functions of the particular combination of self-fertilized parental types and these qualities remain the same whenever the cross is repeated.

(10) The F_1 hybrids are no more variable than the pure strains which enter into them.

(11) The F_2 shows much greater variability than the F_1.

(12) The yield per acre of the F_2 is less than that of the F_1.

We should also like to quote Shull (1911) upon one important point upon which we have but few data:

Necessary corollaries of the view that the degree of vigor is dependent on the degree of hybridity or, in other words, that it is dependent roughly upon the number of heterozygous elements present and not upon any injurious effect of inbreeding per se are (*a*) that when two plants in the same self-fertilized family, or within the same genotype, however distantly the chosen individuals may be related, are bred together there shall be no increase of vigor over that shown by self-fertilized plants in the same genotype, since no new hereditary element is introduced by such a cross; (*b*) that first-generation hybrids produced by crossing individuals belonging to two self-fertilized lines or pure genotypes will show the highest degree of vigor possible in progenies representing combinations of those two genotypes, because in the first generation every individual will be heterozygous with respect to all of the characters which differentiate the two genotypes to which the chosen parents belong, while in subsequent generations recombinations of these characters will increase the average number of heterozygous genes present in each individual; (*c*) that crosses between sibs (sister and brother) among the first-generation hybrids between two genotypes will yield progenies having the same characteristics, the same vigor, and the same degree of heterogeneity as will be shown by the progenies of self-fertilized plants belonging to the same first-generation family.

All of these propositions have now been tested in a limited way. In 1910, nine different self-fertilized families were compared with nine crosses between sibs within the same self-fertilized family; ten crosses between sibs in F_1 families were compared

[1] They are usually as vigorous or more vigorous than the original strains, but may or may not have the original characters. Some characters may have been entirely eliminated.—E. M. E.

with ten self-fertilizations in the same F_1 families; seven families were raised as first-generation hybrids between individuals belonging to different self-fertilized families; and ten families were grown in which self-fertilization had been entirely precluded during the past five years. The average height of plants in decimeters, the average number of rows per ear, and the average yield in bushels per acre in these 55 families are given in the following table:

	Selfed × self.	Selfed × sibs.	F_1.	F_2.	F_1 × self.	F_1 × sibs.	Cross-breds.
Average height	19.28	20.00	25.00	23.42	23.55	23.30	22.95
Average rows	12.28	13.26	14.41	13.67	13.62	13.73	15.13
Average yield	29.04	30.17	68.07	44.62	41.77	47.77	61.52

An examination of this table indicates to me that on the whole my self-fertilized families are not yet quite pure bred; for the sib crosses give on the average a slightly greater height, number of rows per ear, and yield per acre than the corresponding self-fertilized families as shown by a comparison of the first two columns of the table. The same fact is apparent from a comparison of the "F_1 × self" and "F_1 × sibs" columns, except that in this case the heights and number of rows per ear are essentially equal while the yield per acre is significantly higher in the sib crosses than in the self-fertilized families.

These statements should be sufficient to indicate Shull's work and point of view. Other writers have proposed methods designed to utilize commercially the increase in vigor shown by first-generation hybrids, and at least two other theoretical interpretations of this increase have been submitted (Jost, 1907; and Keeble and Pellew, 1910). These papers will be considered later. We will now take up the data obtained in our own experiments.

EXPERIMENTS ON A NORMALLY CROSS-FERTILIZED SPECIES, ZEA MAYS.

EFFECTS OF INBREEDING.

In these experiments over 30 varieties of maize, including all the varieties widely differentiated from each other, have been artificially self-fertilized for from one to seven generations. In every case a loss of vegetative vigor has followed. At least, following the earlier usage, one may say the result is a loss of vigor if it is kept clearly in mind that pathological degeneration is not what is meant. The universal decline in vigor consists simply in a somewhat less rapid cell division or slower growth and a smaller total amount of cell division resulting in smaller plants and plant organs.

Besides this phenomenon, to which there has been no exception, the progeny always become more or less differentiated in normal morphological characters, although this is less marked in some varieties than in others. For example, from the yellow dent variety known

as Leaming various strains differing in the following characters have been isolated during the several generations that they have been inbred:

Red pericarp and colorless pericarp
Red cob and colorless cob.
Red silks and colorless silks.
Red glumes and colorless glumes.
Profusely branched tassels and scantily branched tassels.
Long ears and short ears.
Ears with various numbers of rows.
Ears with large seeds and ears with small seeds.
Ears with straight rows and ears with crooked rows
Ears high on the stalk and ears low on the stalk.
Stalks with many tillers and stalks with few tillers.

Other minor differences have been observed, but these will serve to show just what is meant by "normal differences." There were also differences in yield of seed—described later in this bulletin—some of which may not seem to be normal in character at first thought, but which we hope to show are not less normal than those given above.

Besides these variations, aberrant individuals appeared in a few strains with characters which might well be called abnormal; that is, they are monstrous characters. But this does not mean that they might not have originated in the same manner as normal characters, for they are transmitted as such. Two of these characters, fasciated ears and bifurcated cobs, show a simple Mendelian segregation with incomplete dominance; two others, striped leaves and dwarf plants, are probably recessives. It is possible, however, that one form of striped leaf is the heterozygote between pure white and normal green. It may be that the first two of these abnormalities are not simply isolated as Mendelian segregates. They have also appeared in commercial varieties grown on very fertile soil, a fact that suggests their origin through interference with normal processes of cell division, acceleration in one case and retardation in the other.

The variability of the strains in the above characters decreased as inbreeding was continued, until after four generations they were practically constant for all grosser characters. This does not mean that physiological fluctuation was not as great as in the original strain. It was not reduced in the least degree. Nor can it be said that no new heritable variations arose. Certain variations did appear which may have been new to the strain—witness the fasciated ears—but of this one could not be certain. Furthermore, it is not meant that after four or five generations of inbreeding a type is homozygous in all of its characters. Such a gametic condition is theoretical and could never be recognized in a pedigree culture. But near homozygotes or

near homozygous genotypes are obtained without selection simply by inbreeding. The reason for this is simple.

Mendel in his original paper showed that if equal fertility of all plants in all generations is assumed and, furthermore, if every plant is always self-fertilized then in the n^{th} generation the ratio of any particular allelomorphic pair (A, a) would be $2^n-1\ AA:2\ A\ a:2^n-1\ a\ a$. If we consider only homozygotes and heterozygotes, the ratio is $2^n-1:1$. Of course the matter is not quite so simple when several allelomorphs are concerned, but in the end the result is similar. Heterozygotes are eliminated and homozygotes remain. The probable number of homozygotes and any particular class of heterozygotes in any generation r is found by expanding the binomial $[1+(2^r-1)]^n$ where n represents the number of character pairs involved. The exponent of the first term gives the number of heterozygous and the exponent of the second term the number of homozygous characters. As an example, suppose we desire to know the probable character of the fifth segregating generation (F_6) when inbred, if three character pairs are concerned. Expanded we get

$$1^3+3[1^2(31)]+3[1(31)^2]+(31)^3.$$

Reducing, we have a probable fifth-generation population consisting of 1 heterozygous for three pairs; 93 heterozygous for two pairs; 2,883 heterozygous for one pair; 29,791 homozygous in all three character combinations.

From this illustration we think it is fairly easy to see that no matter in how many characters a plant is heterozygous, continued inbreeding will sooner or later eliminate them. Close selection, of course, tends toward the same end, but not with the rapidity or certainty of self-fertilization.

Inbreeding a naturally crossbred plant, then, has these results:

(1) There is partial loss of power of development, causing a reduction in the rapidity and amount of cell division. This phenomenon is universal and therefore can not be related to inheritance. Further, it continues only to a certain point and is in no sense an actual degeneration.

(2) There is an isolation of subvarieties differing in morphological characters accompanying the loss of vigor.

(3) There is often regression away from instead of toward the mean of the general population.

(4) As these subvarieties become more constant in their characters the loss of vigor ceases to be noticeable.

(5) Normal strains with such hereditary characters that they may be called degenerate strains are sometimes, though rarely, isolated.

(6) It is possible that pure strains may be isolated that are so lacking in vigor that the mechanism of cell division does not properly perform its function, and abnormalities are thereby produced.

The maize families shown in Table I illustrate some of these facts, if the yield of shelled corn per acre is taken as a basis of comparison of vigor. These families are not selected to fit a theory, but include representatives of four of the great subdivisions of the species out of those grown in sufficient quantity to give considerable confidence in the determination of yield. Many other types have been inbred for from one to four years, but neither land nor time was available to grow them in large quantities. Their behavior, however, was the same. Inbreeding always reduced the yield of seed and the height and delayed the time of flowering. In general, the decrease in vigor lessened with the inbreeding. Further, both good and bad strains were isolated.

TABLE I.—*Effect of inbreeding on the yield of maize.*

Variety.	Year grown.	Number of years inbred.	Yield in bushels per acre.	Variety.	Year grown.	Number of years inbred.	Yield in bushels per acre.
Watson's flint No. 5	1908	.....	75.7	Leaming dent—Contd.			
No. 5-8	1909	1	47.5	No. 1-7-1-1	1908	3	46.0
No. 5-8-3	1910	2	36.1	No. 1-7-1-1-1	1910	4	63.2
Starchy No. 10 [1]	1908	.....	70.5	No. 1-7-1-1-1-4	1911	5	25.4
No. 10-3	1909	1	56.0	No. 1-7-1-2	1909	3	59.7
No. 10-3-7	1910	2	67.0	No. 1-7-1-2-2	1910	4	68.1
No. 10-3-7-3	1911	3	39.1	No. 1-7-1-2-2-9	1911	5	41.3
No. 10-4	1909	1	43.0	Leaming dent No. 1	1905	.....	88.0
No. 10-4-8	1910	2	48.7	No. 1-9	1906	1	42.3
No. 10-4-8-3	1911	3	29.3	No. 1-9-1	1908	2	51.7
Stowell's sweet No. 19	1909	.....	93.2	No. 1-9-1-2	1909	3	35.4
No. 19-4	1910	1	58.7	No. 1-9-1-2-4	1910	4	47.7
No. 19-4-7 [2]	1911	2	51.2	No. 1-9-1-2-4-6	1911	5	26.0
No. 19-8	1910	1	53.6	Leaming dent No. 1	1905	.....	88.0
No. 19-8-2 [2]	1911	2	42.1	No. 1-12	1906	1	38.1
Leaming dent No. 1	1905	.....	88.0	No. 1-12-1	1907	2	32.8
No. 1-6	1906	1	59.1	No. 1-12-1-1 [1]	1908	3	46.2
No. 1-6-1	1908	2	95.2	No. 1-12-1-1-2	1909	4	23.3
No. 1-6-1-3	1909	3	57.9	No. 1-12-1-1-2-4	1910	5	16.5
No. 1-6-1-3-4	1910	4	80.0	No. 1-12-1-1-2-4-11	1911	6	2.0
No. 1-6-1-3-4-4	1911	5	27.7	No. 1-12-1-1-4	1909	4	28.7
Leaming dent No. 1	1905	.....	88.0	No. 1-12-1-1-4-14	1910	5	9.5
No. 1-7	1906	1	60.9	No. 1-12-1-1-4-14-3	1911	6	2.0
No. 1-7-1 [1]	1907	2	59.3				

[1] Two selections from the progeny of this ear grown.
[2] Probably a normal yield. Grown on a more fertile soil than the rest in 1911.

The different families were all planted on the same plat under uniform conditions each season, but, unfortunately, circumstances made it necessary to grow them upon different fields each season. It is therefore necessary to take into consideration the differences in soil fertility and meteorological conditions each year to see the truth of our conclusions, namely, that continued inbreeding caused only isolation of strains of varying potency. The greatest differences in the environmental conditions were in the years 1908, 1909, and 1911.

In 1908 the land used was highly fertile and the general environmental conditions much above the normal. Four stalks per hill were grown this season, but as only three were grown in other years the actual yields have been reduced one-fourth. Even at this disadvantage the yields in 1908 are probably somewhat high. For opposite reasons, poor soil and badly distributed rainfall, the yields of 1909 are somewhat too low and the yields of 1911 are very much too low. This will be appreciated if the low yields for 1911 are examined in Table III.

Since the data on the Leaming dent variety are the most interesting they are repeated in a somewhat different form in Table II. There they are shown in a regular line of descent.

TABLE II.—*Effect of inbreeding on a variety of Leaming dent maize.*

(Yield, in bushels, of shelled corn per acre.)

Parent variety.	Generations inbred and years in which grown.					
	1	2	3	4	5	6
88.0 (1905)....	59.1 (1906)	95.2 (1908)	57.9 (1909)	80.0 (1910)	27.7 (1911)	.....
	60.9 (1906)	59.3 (1907)	46.0 (1908)	63.2 (1910)	25.4 (1911)	.....
			59.7 (1909)	68.1 (1910)	41.3 (1911)	.....
	42.3 (1906)	51.7 (1908)	35.4 (1909)	47.7 (1910)	26.0 (1911)	.....
	38.1 (1906)	32.8 (1907)	46.2 (1908)	23.3 (1909)	16.5 (1910)	2.0 (1911)
				28.7 (1909)	9.5 (1910)	2.0 (1911)

The Leaming, a well-known commercial dent variety, yielded 88 bushels per acre the year before it was first inbred. The season was normal, and this yield may be considered fairly typical of what the variety will do on a moderately good soil. Four ears were inbred and were grown in 1906. This was again an average year. The four strains showed marked decreases in yield and notable differences in their characters. The year 1907 was again an average year, and the second inbred generations are normal. Two strains were not grown as second inbred generations until 1908, however, and they are therefore too high. In 1909 the yields are too low; in 1910 normal, and in 1911 much too low. With these facts in mind, an examination of the tables shows how the strains became more and more differentiated. The first strain, No. 6, is a remarkably good variety of corn even after five generations of inbreeding. It yielded 80 bushels per acre in 1910. The yield was low in 1911, but since all yields were low that year it can

hardly be doubted that this strain will continue to produce good normal yields of grain. In the field, even in 1911, the plants were uniformly vigorous and healthy and were especially remarkable for their low variability. The poorest strain, No. 12, is partially sterile, never fills out at the tip of the ear and can hardly exist alone. In 1911 it yielded scarcely any corn but will no doubt continue its existence as a partly sterile variety. Plate I shows ears and tassels of an almost sterile strain isolated by inbreeding.

CROSSING INBRED TYPES.

When two of these inbred strains are again crossed, the F_1 generation shows an immediate return to normal vigor. The plants are earlier and taller, and there is a greater total amount of dry matter per plant. For example, in 1911 the average height of all the strains of inbred Leaming dent was 84 inches while the average height of the 16 hybrid combinations was 111 inches and the height of the shortest hybrid combination was 1 foot greater than that of the tallest inbred strain.

Table III gives the yields of shelled corn per acre of several inbred types, together with the yields of many first-generation crosses. Many interesting points may be learned from this table, provided it is remembered that maize is greatly influenced by environmental conditions and therefore only populations grown in the same season should be compared with each other. For this reason the comparisons between first-generation hybrids and the unselected commercial types from which the inbred strains came are not to be given too great weight. On the other hand, there is such an enormous difference between many of the first-generation hybrids and the normal commercial varieties that the conclusion that the former are often better is perfectly just.

TABLE III.—*Comparative yields of inbred types of maize and their first-generation crosses.*

Variety.	Year grown.	Number of years inbred.	Yield in bushels per acre.	Comparison between F_1 and unselected commercial strains.
White dent No. 8	1908	.....	121.0	121.0
Leaming dent No. 1-7	1908	3	62.0	88.0
No. (8×1-7), F_1	1908	.....	142.0	142.0
Flint No. 5	1909	1	47.5	75.7
Flint No. 11	1909	1	44.2	48.0
No. (5×11), F_1	1909	.....	76.3	76.3
Flint No. 5	1909	1	47.5	75.7
Leaming dent No. 1-6	1909	3	57.9	88.0
No. (5×1-6), F_1	1909	.....	88.9	88.9
No. (5×1-6), F_1	1910	.....	105.5	105.5
No. (5×1-6)-1, F_2	1910	1	54.1	54.1
No. (5×1-6)-2, F_2	1910	1	48.9	48.9
Starchy No. 10	1910	2	48.7	70.5
Leaming dent No. 1-6	1910	4	80.4	88.0
No. (10×1-6), F_1	1910	.....	139.0	139.0

TASSELS AND EARS OF AN ALMOST STERILE STRAIN OF CORN ISOLATED BY INBREEDING.

(Photographed by Emerson.)

WATSON'S FLINT AND LONGFELLOW FLINT CORN INBRED TWO YEARS WITH F_1 HYBRID.

(All ears hand-pollinated.)

TABLE III.—*Comparative yields of inbred types of maize and their first-generation crosses*—Continued.

Variety.	Year grown.	Number of years inbred.	Yield in bushels per acre.	Comparison between F_1 and unselected commercial strains.
Leaming dent No. 1-7	1910	4	65.5	88.0
Sweet No. 19	1910	1	53.6	93.2
No. (1-7×19), F_1	1910	.....	142.7	142.7
Leaming dent No. 1-9	1909	4	23.3	88.0
Leaming dent No. 1-12	1909	3	35.4	88.0
No. (1-12×1-9), F_1	1909	.....	110.2	110.2
No. (1-12×1-9), F_1	1910	.....	117.5	117.5
No. (1-12×1-9)-1, F_2	1910	1	102.2	102.2
No. (1-12×1-9)-4, F_2	1910	1	91.5	91.5
No. (1-12×1-9)-12, F_2	1910	1	91.5	91.5
Leaming dent 1-6	1911	5	27.7	.....
Leaming dent 1-7-1	1911	5	25.4	.....
Leaming dent 1-7-2	1911	5	41.3	.....
Leaming dent 1-9-2	1911	5	26.0	.....
Leaming dent 1-12-2	1911	6	2.0	.....
Leaming dent 1-12-4	1911	6	2.0	.....
No. (1-6×1-7-1), F_1	1911	.....	75.6	.....
No. (1-6×1-7-2), F_1	1911	.....	58.3	.....
No. (1-6×1-9-2), F_1	1911	.....	31.6	.....
No. (1-6×1-12-2), F_1	1911	.....	10.2	.....
No. (1-7-1×1-6), F_1	1911	.....	58.8	.....
No. (1-7-1×1-7-2), F_1	1911	.....	41.3	.....
No. (1-7-1×1-9-2), F_1	1911	.....	51.5	.....
No. (1-7-1×1-12-2), F_1	1911	.....	16.9	.....
No. (1-7-1×1-12-4), F_1	1911	.....	60.2	.....
No. (1-7-2×1-6), F_1	1911	.....	57.7	.....
No. (1-7-2×1-12-2), F_1	1911	.....	63.5	.....
No. (1-9-2×1-6), F_1	1911	.....	37.3	.....
No. (1-9-2×1-7-1), F_1	1911	.....	46.2	.....
No. (1-9-2×1-12-2), F_1	1911	.....	3.6	.....
No. (1-12-2×1-7-2), F_1	1911	.....	76.9	.....
No. (1-12-2×1-12-4), F_1	1911	.....	24.5	.....

Attention is called first to the fact that in combinations (5×1–6) and (1–12×1–9) both the first and second hybrid generations are grown in the same year. The first hybrid generation gives an enormous increase over the inbred types. The second hybrid generation is also much greater than the inbred strains, but recombination with the production of homozygotes has taken place, and this generation gives much lower yields than when the greatest possible heterozygosity existed as in the first hybrid generation.

Attention should next be directed to the results of 1911, when nearly all the possible combinations of the inbred Leaming strains were made. The yields of the inbred types given are those with one more year of inbreeding than the real parents of the first-generation hybrids. But considering the amount of previous inbreeding to which they had been subjected this probably makes but little difference. As stated before, the yields in 1911 were very much reduced by the unfavorable season, and this too must be given due weight in studying the yields. As a whole the combinations into which No. 1–7 was introduced were the best while those into which the poor type No. 12 was introduced are the poorest. The combination (1–7–1×1–12–4) was, however, a very good cross.

Possibly a question may arise as to whether the fine yields of the combination (1–12×1–9) in 1909 and 1910 and the poor yields of combination (1–9–2×1–12–2) in 1911 are not due to a difference in the behavior of a reciprocal cross. This is probably not the correct reason, for in general there is no difference in reciprocals. No. 1–12 was further inbred when the combinations grown in 1911 were made and this is probably the cause of their poor showing. In the earlier combination, No. 1–12 undoubtedly had a somewhat different gametic constitution than when the later crosses were made. Some essential factor may have been eliminated, therefore, during the further inbreeding. On the other hand, the whole explanation may lie in the poor season of 1911.

The marked increase in productiveness of the F_1 hybrid over the parent inbred types of maize is well shown in Plates II and III, while Plate IV illustrates the falling off in productiveness of the F_2 generation as compared with the F_1 generation from inbred types. Plate V serves to show the striking increase in vigor of the F_1 generation from a cross of pure lines.

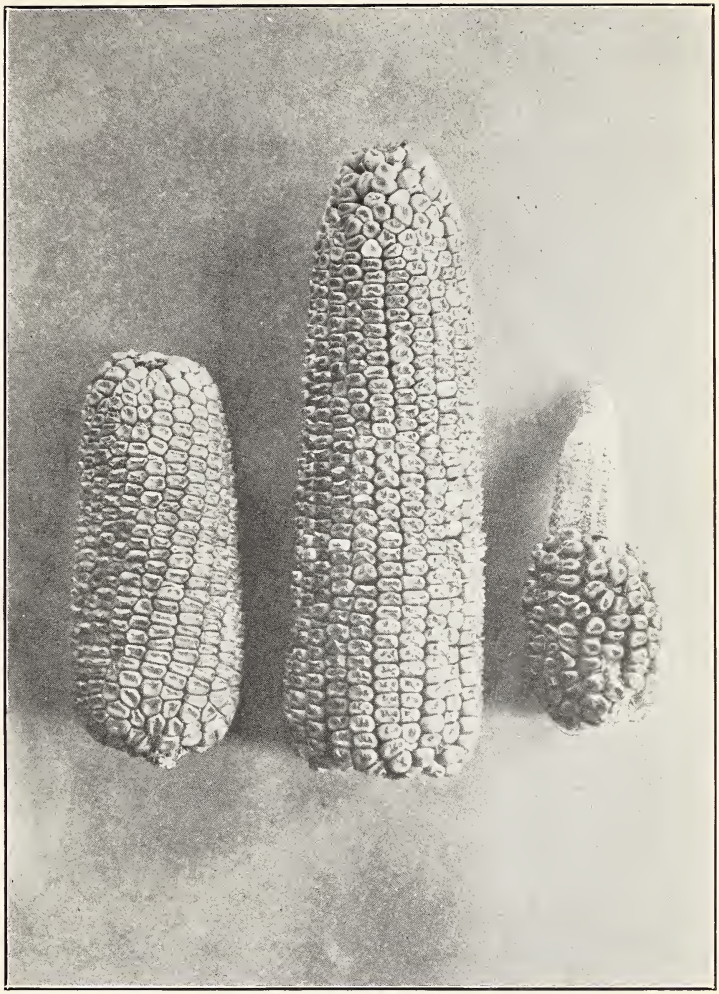
The logical conclusion from the facts brought out above is apparently that good inbred strains are better than poor ones in combination, but that good and poor strains crossed together may give very strong plants.

EXPERIMENTS ON SPECIES GENERALLY SELF-FERTILIZED.

As experimental material that contrasts well with maize, the genus Nicotiana was selected. This genus contains a large number of species and varieties, most of which have flowers adapted to self-fertilization. No doubt cross-fertilization sometimes occurs in most of them, but it is not the rule.

Seeds of several species and many varieties were obtained from various parts of the world through the kindness of a number of friends. The same species did not always arrive with the same name, and we have not been fortunate enough to have the aid of a Nicotiana specialist in their identification. We have, however, studied them in pure-line cultures during the past four years and have compared them with specimens in the Gray Herbarium of Harvard University. This gives us some confidence that the names used are in accord with the species as accepted and described by Comes in his "Monographie du Genre Nicotiana," Naples, 1899.

Many crosses have been made between different varieties within the two species *Nicotiana tabacum*, L., and *N. rustica*, L. Some of the varieties of *N. tabacum* have been practically identical as far as external appearance is concerned, although received under different names. When this has been the case, the results have been varied.



LEAMING DENT STRAINS OF CORN, NO. 9 (AT LEFT) AND NO. 12 (AT RIGHT), AFTER FOUR YEARS' INBREEDING, COMPARED WITH F_1 HYBRID (IN CENTER).

(All ears hand-pollinated.)

INBRED STRAINS OF LEAMING DENT CORN COMPARED WITH F_1 AND F_2 GENERATIONS.

(Grown in 1910.)

STRAINS 6 AND 7 OF LEAMING PURE LINES OF CORN (AT LEFT) AND F_1 GENERATION OF CROSSES 6 × 7-1 AND 6 × 7-2 (AT RIGHT).

(Grown in 1911.)

FIG. 1.—NICOTIANA TABACUM VARIETY.

($\frac{1}{22}$ natural size.)

FIG. 2.—NICOTIANA TABACUM VARIETY × N. SILVESTRIS, F_1 GENERATION.

($\frac{1}{30}$ natural size.)

FIG. 3.—NICOTIANA SILVESTRIS.

($\frac{1}{15}$ natural size.)

For example, two exceedingly similar varieties may give hybrids with no greater luxuriance of growth than the pure parent strains; other varieties as similar in appearance may give hybrids with as much as 25 per cent greater vigor than the average of the two parents. In this case the criterion of greater vigor is height of plant. If one accepts the old view that nonrelationship between the individuals used as parents is the reason for the increased vigor of the hybrids, there would be no logical reason why all such crosses should not show the same condition. If, on the other hand, the correct explanation is to be sought in the similarity or dissimilarity of the gametic constitution of the parents, it is quite evident that different crosses among varieties similar in external characters may behave in a different manner. Plants having a close genetic relationship with each other—that is, descendants of a previous cross—may be quite different in gametic constitution and therefore show an increased vigor in the F_1 hybrid; but genetically unrelated plants of practically the same gametic constitution may be obtained from different parts of the world under different names and not be expected to show an increased vigor in the hybrid.

An example of the amount of increase in height in crosses between *Nicotiana rustica brazilia* Comes and *N. rustica scabra* Comes, both obtained from Italy, is shown in Table IV.

TABLE IV.—*Height of crosses between Nicotiana rustica scabra (352) and N. rustica brazilia (349).*

Variety or cross.	Class centers in inches.																		
	24	27	30	33	36	39	42	45	48	51	54	57	60	63	66	69	72	75	78
349	4	10	22	14	7	....	....	....	....	....	....	....	....	....	....	....	....	....	....
352	....	....	....	....	....	....	2	1	5	11	16	17	6	....	....	....	....	....	....
352 × 349 F_1	....	....	....	....	....	....	....	....	....	1	3	0	5	5	5	6	1	1	....
349 × 352 F_1	....	....	....	....	....	....	....	....	....	....	....	3	5	2	4	6	5	1	2

The reciprocal crosses both showed a marked tendency to advance the mode until in each case it is higher than the highest plant of the taller parent. Different strains of *N. tabacum* var. "Sumatra," of *N. tabacum* var. "Havana," and of *N. rustica* var. brazilia, identical in external appearance, obtained both from the same locality and from opposite parts of the world, have also shown increased height when crossed. On the other hand, strains of *N. tabacum* varieties "Sumatra" and "Havana," from seed of plants grown in Connecticut, when crossed with like varieties from seed of plants grown in Italy have shown no increase in vigor. Accounts of other similar crosses could be given, but it seems unnecessary to multiply examples. We will therefore pass to a consideration of the specific crosses shown in Table V.

TABLE V.—*Condition of hybrids in crosses between species of Nicotiana.*

Cross.	Germination.	Fertility.	Condition of hybrid.
	Per cent.		
N. alata Lk. and Otto, var. grandiflora Comes:			
× N. forgetiana Hort. (Sand.)	100	Fertile	25 per cent in height; very vigorous and profuse in flowers.
× N. langsdorffii Weinm	100	...do	105 per cent in height; vigorous and profuse in flowers.
× N. longiflora Cav	100	Sterile	100 per cent in height; 100 per cent in vigor.
× N. paniculata L	2	Slightly fertile.	80 per cent in height; 80 per cent in general vigor.
× N. tabacum L	3	Sterile(?)	Very weak; seedlings died.
N. bigelovii Wats.:			
× N. alata grandiflora Comes	0		
× N. longiflora Cav	0		
× N. quadrivalvis Pursh	100	Fertile	125 per cent in height; 100 per cent in general vigor.
× N. silvestris Speg. and Comes	0		
× N. tabacum L	100	Sterile	120 per cent in height; 120 per cent in vigor; profuse in flowers.
N. forgetiana Hort. (Sand.):			
× N. alata grandiflora Comes	100	Fertile	125 per cent in height; 130 per cent in general vigor; profuse in flowers.
× N. langsdorffii Weinm	100	...do	160 per cent in height; 125 per cent in general vigor; profuse in flowers.
× N. tabacum L	0		
N. glauca Grah. × N. tabacum L	60	Sterile	80 per cent in height; less vigorous.
N. glutinosa L. × N. tabacum L	(?)		Gärtner obtained plants higher and more vigorous than parents.
N. langsdorffii Weinm.:			
× N. alata Lk. Otto, var. grandiflora Comes.	100	Fertile	105 per cent in height; 100 per cent in vigor.
× N. bigelovii Wats	100	Sterile	110 per cent in height; very vigorous.
× N. forgetiana Hort. (Sand.)	100	Fertile	110 per cent in height; 100 per cent in vigor; profuse in flowers.
× N. paniculata L	0		
N. longiflora Cav. × N. alata Lk. and Otto, var. grandiflora Comes.	100	Sterile	100 per cent in height and general vigor.
N. paniculata L.:			
× alata Lk. and Otto, var. grandiflora Comes.	25	Slightly fertile.	95 per cent in height; rather weak.
× N. bigelovii Wats	2	Sterile	100 per cent in height; 95 per cent in vigor.
× N. langsdorffii Weinm	5	...do	15 per cent in height; very weak and stunted.
× N. longiflora Cav	0		
× N. rustica L	100	Partially fertile.	125 per cent in height; very vigorous and profuse in flowers.
× N. tabacum L	5	Sterile(?)	Plants very weak and small.
N. plumbaginifolia Viv. × N. longiflora Cav.	100	Fertile	125 per cent in height; 110 per cent in general vigor.
N. quadrivalvis Pursh. × N. bigelovii Wats.	100	...do	110 per cent in height; 100 per cent in general vigor; profuse in flowers.
N. rustica L.:			
× N. alata Lk. and Otto, var. grandiflora Comes.	5		So weak that plants lived only about two weeks.
× N. langsdorffii Weinm	80	Sterile(?)	110 per cent in height; 110 per cent in vigor; very profuse in flowers.
× N. paniculata L	100	Partially fertile.	125 per cent in height; very vigorous; profuse in flowers.
× N. tabacum L	5	Sterile	180 per cent in height; extremely vigorous; profuse in flowers.
N. silvestris Speg. and Comes:			
× N. longiflora Cav	0		
× N. tabacum L	100	Sterile	140 per cent in height; 120 per cent in vigor; profuse in flowers.
N. tabacum L.:			
× N. alata Lk. and Otto, var. grandiflora Comes.	2	...do	10 per cent of average of parents in height and in general vigor.
× N. bigelovii Wats	100	Almost sterile.	120 per cent of average of parents in height and in general vigor.
× N. glauca Grah	60	Sterile	85 per cent of average of parents in height and 80 per cent in general vigor.
× N. glutinosa L	5		25 per cent of average of parents in height; Gärtner obtained plants more vigorous than parents.
× N. langsdorffii Weinm	10	...do	60 per cent of average of parents in height; 75 per cent in general vigor.
× N. longiflora Cav	0		
× N. paniculata L	1		Very small and weak; died before flowering.
× N. plumbaginifolia Viv	0		
× N. silvestris Speg. and Comes	100	Sterile	135 per cent of average of parents in height; 120 per cent in vigor; profuse in flowers.

FIG. 1.—NICOTIANA RUSTICA TEXANA.

($\frac{1}{8}$ natural size.)

FIG. 2.—NICOTIANA RUSTICA TEXANA × N. TABACUM VARIETY, F1 GENERATION.

($\frac{1}{26}$ natural size.)

FIG. 3.—NICOTIANA TABACUM VARIETY.

($\frac{1}{18}$ natural size.)

FIG. 1.—NICOTIANA ALATA GRANDIFLORA.

(Twice scale of figure 2.)

FIG. 2.—NICOTIANA TABACUM VARIETY.

FIG. 3.—NICOTIANA ALATA GRANDIFLORA × N. TABACUM. WEAK AND STERILE HYBRID.

(Six times scale of figure 2.)

The voluminous data that have been collected on these hybrids have been condensed and approximated so that they include only facts germane to the matter in hand. Those crosses designated as not having germinated are crosses in which seed was obtained, but from which no plant was obtained from a planting of at least one hundred seeds. In some of these crosses the seed was poorly formed (without embryo) and one may say conclusively that they would never produce plants. Other crosses gave fully mature, perfect seed which did not germinate. Possibly the proper conditions for their germination were not obtained. At least it would be rash to conclude that all of the crosses of which the seed did not germinate would never produce plants under any conditions. But it is proper to say that some crosses are possible in which the hybrid plant reaches no further than the seed stage. A few hybrids, viz, *Nicotiana tabacum* × *N. paniculata*, *N. rustica* × *N. alata grandiflora*, etc., germinated and produced a few weak plants that died before flowering. There were still others that produced mature plants, but plants shorter than either parent and weak in character. By far the majority of the hybrids, however, were taller than the average of the parents and many were taller than either parent. The luxuriance of their growth was also such that they may be said to be more vigorous than either parent. Plate VI shows the result of a cross between *Nicotiana tabacum*, var., and *Nicotiana silvestris*.

One gets the idea from a survey of the crosses in this genus that there are (*a*) plants so different that they will not cross; (*b*) crosses that produce seed that contain no proper embryo; (*c*) crosses that produce seed with embryo, but which go no further than the resting stage of the seed; (*d*) crosses less vigorous than either parent; (*e*) crosses more vigorous than the average of the parents; and (*f*) crosses more vigorous than either parent. It seems probable, then, that actual fusion may take place between gametes either so different in character that the zygote can not develop or in which the male cell does not bring in the proper substance to stimulate development. On the other hand, when development does take place in a normal manner the great majority of cases show a stimulus greater in the hybrids than in the pure species. Compare Plate VII.

It might be supposed that the luxuriant development of many of these hybrids is due to their sterility, that is, due to the fact that no energy is used in seed formation. Such an idea was held by some of the earlier hybridizers, but was disproved by Gärtner. Nor is it justified by our own experience. Fertile crosses between plants differing in character either equal or exceed the parental vigor; sterile crosses may show a great increase in vigor or they may show a great diminution in vigor. Plate VIII represents a sterile hybrid

showing decided diminution in vigor. But there need be no confusion in the interpretation of these facts. It is known that some plants are so unlike that there is mechanical or chemical obstruction to fertilization. In one case the stigmatic fluid may be poisonous to certain foreign pollen; in another case the pollen tubes can not penetrate the micropyle; sometimes nuclei do not enter the micropyle; frequently the two nuclei will not fuse. Such conditions absolutely prevent a cross. On the other hand, where crossing is possible, all of the physiological processes normal to the plant may not be carried out. The difficulty often lies in the maturation of the sex cells, the reduction of the chromatin, and the preparation for a new sexual act. In the proposed parent plants this has already taken place naturally. The male and female gametes are ready for fusion, and if nothing interferes this fusion takes place. But this does not mean that normal development can take place. Cell division may be so difficult that no embryo is formed, there being simply a pericarp formed by the reaction of maternal tissue to stimulation. Again, development of the embryo may take place, primarily because the difficulty of development is decreased through the nutrition furnished by the mother plant. But it may stop at this point. Thus it is obvious that where the parent plants are so different that normal somatic cell division can not take place, weak plants result even though they are heterozygous for many characters. If, however, cell division is normal we may believe that the vigor of the hybrid increases directly with the amount or the kind of heterozygosis present, without regard to whether the plant is sterile or fertile. Sterility, therefore, is often simply an inability to mature the sex elements properly, possibly because of mechanical obstruction to normal reduction of chromosomes differing widely in their character, and sometimes it is correlated with abnormal ontogeny.

We make the statement that hybrid vigor increases with the amount or with the kind of heterozygosis advisedly. The increased vigor may vary roughly with the number of heterozygous characters present, up to that limiting case where the action of other forces prevents or obscures their influence, or it may depend largely upon the quality of the characters that are heterozygous. This matter has not been determined; in reality it makes no difference with the thesis under discussion. It is an interesting problem, but can hardly be tested experimentally by crossing owing to the number of unknown characters that may be present in either a heterozygous or homozygous condition. The proof submitted here rests entirely upon the effects obtained by continued inbreeding as explained by the mathematical expectancy of homozygotes and heterozygotes under continued inbreeding.

One further point ought to be noted here. It has been shown that weak types are sometimes isolated from maize by inbreeding, their delicate constitution being due, it is assumed, to homozygosis of heritable characters that produce weakness and not to the mere fact of inbreeding. Does one obtain weak types in self-fertilized species? Undoubtedly such strains arise, but it is difficult to obtain examples because the weakness of individual plants is usually a physiological fluctuation due to external conditions and is not transmitted. This has been found to be true by growing seedlings from weak plants that have been self-fertilized. They usually give normal plants. Weak strains have been isolated, however, from *Nicotiana tabacum*, from *N. paniculata*, and from *N. attenuata* that continued to transmit their poor constitution. We may conclude, therefore, that weak strains arise in self-fertilized species, but are eliminated by natural selection.

THE CHARACTERS AFFECTED BY HETEROZYGOSIS

The term vigor has hitherto been used with the general meaning which the biologist readily understands. We will now endeavor to show in what plant characters this vigor finds expression. It is not an easy task because of the possibility of confusing the phenomenon of Mendelian dominance with the physiological effect due to heterozygosis. The confusion is due to a superficial resemblance only. Dominance is the expressed potency of a character in a cross and affects the character as a whole. A morphological character like the pods of individual maize seeds, or the product of some physiological reaction like the red color of the seed pericarp in maize may be perfectly dominant, that is, it may be developed completely when obtained from only one parent. Size characters on the other hand usually lack dominance or at best show incomplete dominance. The vigor of the first hybrid generation theoretically has nothing to do with these facts. This is easily demonstrated if one remembers that the increased vigor manifested as height in the F_1 generation can not be obtained as a pure homozygous Mendelian segregate, which would be possible if due to dominance. Furthermore, the universality with which vigor of heterozygosis is expressed as height shows the distinction between the two phenomena. If the greater height were the expression of the meeting of two factors ($T_1t_2 \times t_1T_2$) both of which were necessary to produce the character, one could not account for the frequency of the occurrence. Nevertheless, in practice the confusion exists, and while we have considerable confidence in the conclusions drawn from our experiments, we have no intention of expressing them dogmatically.

It has been stated that the vigor due to heterozygosis is primarily an increase and an acceleration of cell division; in other words, an

increased power of assimilation. This is first of all expressed by the increased size of the root system, a fact noticed by Kölreuter and Gärtner as quoted on page 9. This is the first noticeable difference, for the size of the cotyledons of the hybrid is largely influenced by the size of the maternal pericarp, yet there is a slight increase in the cotyledon size, as we have found in experiments with species of the genus Impatiens and with the tomato, *Lycopersicum esculentum*. Hybrid seedlings next show the increased vigor by their rapidity of growth tending toward an earlier maturity. This feature is the acceleration of cell division referred to above. Ultimately, however, there is not only acceleration but increased cell division, resulting in taller plants. Data supporting this fact have already been shown in papers on maize (East, 1911, 1911a). The increased size is entirely internodal. Neither in crosses between maize varieties nor between varieties of *Nicotiana tabacum* is there any tendency to increase the number of nodes. This stem growth is comparatively much greater than is increased leaf surface in the plants investigated (*N. tabacum*), although the latter can be definitely traced.

The size of the flower is not affected, at least not certainly. The fruit also does not seem to be affected where there is a small natural amount of cell division, as in the capsule of tobacco. In fleshy fruits like the tomato or eggplant there is a marked increase. This is probably true also of the large pomes and pepos, but this is only a surmise by analogy.

The increased vigor of the whole plant makes it possible for more flowers and fruit to be produced, as we have determined in tobacco and tomato. A more or less indeterminate inflorescence is always prolonged, which probably accounts for the increased size that is found in the ears of maize hybrids.

There are many less important plant characters upon which no data have been gathered, but the action of heterozygosis is known well enough to justify the former statement that it affects the amount and rapidity of assimilation as expressed by cell division.

THEORETICAL INTERPRETATION OF RESULTS.

At this point it may be well to stop, collect our facts, and discuss their theoretical interpretation, notwithstanding a certain repetition it will involve. We believe it to be established that—

(1) The decrease in vigor due to inbreeding naturally cross-fertilized species and the increase in vigor due to crossing naturally self-fertilized species are manifestations of one phenomenon. This phenomenon is heterozygosis. Crossing produces heterozygosis in all characters by which the parent plants differ. Inbreeding tends to produce homozygosis automatically.

(2) The phenomenon exists and is in fact widespread in the vegetable kingdom.

(3) Inbreeding is not injurious in itself, but weak types kept in existence in a cross-fertilized species through heterozygosis may be isolated by its means. Weak types appear in self-fertilized species, but are eliminated because they must stand or fall by their own merits.

The logical interpretation of all of these facts rests, we believe, on the acceptance of Johannsen's (1903, 1909) "genotype conception of heredity." This conception in turn is an extension of Weismannism[1] without Weismann's mechanistic speculations, supported by Mendelism. Johannsen (1911) gives the essential points of this conception in these paragraphs:

> The personal qualities of any individual organism do not at all cause the qualities of its offspring, but the qualities of both ancestor and descendant are in quite the same manner determined by the nature of the "sexual substances"—i. e., the gametes—from which they have developed. Personal qualities are then the reactions of the gametes joining to form a zygote; but the nature of the gametes is not determined by the personal qualities of the parents or ancestors in question. This is the modern view of heredity.
>
> The main result of all true analytical experiments in questions concerning genetics is the upsetting of the transmission conception of heredity, and the two different ways of genetic research, pure-line breeding as well as hybridization after Mendel's model, have in that respect led to the same point of view, the "*genotype conception*" as we may call the conception of heredity just now sketched.

A simple illustration of what is meant by the above statement is as follows: Suppose a maize with red pericarp (*RR*) be crossed with one with a colorless pericarp (*rr*). In the hybrid the gametes *R* and *r* are formed in equal quantities. By chance mating $1RR:2Rr:1rr$ are obtained. Now the homozygous dominant *RR* is exactly like the heterozygote *Rr* in appearance, but the one breeds true to red pericarp and the other again throws about 25 per cent white progeny. In other words, the gametic composition of the zygotes determines whether the resulting plants shall have ears with red or with colorless pericarps, but the fact that a plant has an ear with a red pericarp does not show what kind of gametes it will form.

The genotype conception of heredity, as stated before, rests on the noninheritance of somatic modifications and the general truth of Mendelism. The first part of the proposition now has almost universal support. All data point to a germ-cell-to-germ-cell hereditary transmission. In certain animals it has been demonstrated that there is an early segregation or setting apart of the material designed

[1] One need become a Weismannian only so far as to agree with the observed facts which have shown that the transmission of acquired characters must be so relatively infrequent as to make the possibility negligible in experimental genetics and plant breeding.

to become the germ cells. This fact naturally has been proved in but few animals, but from it one must infer that in all metazoa there is a relative independence of soma and germ plasm undreamed of a few decades ago. In the higher plants no visible difference between germ plasm and soma plasm has been proved, yet the recent experiments of Baur and of Winkler on periclinal chimeras or false-graft hybrids have shown that one of the subepidermal layers is probably alone responsible for the sexual cells. In recent years few biologists have believed that surrounding conditions did not occasionally modify gametic structures. On the other hand, fewer and fewer investigators have maintained that any sort of somatic adaptation would impress the germ plasm with the ability to transmit the same modification.

The experimental work on the genotype conception of heredity has been largely a demonstration of the last statement. It has shown that in general fluctuations caused by ordinary environmental changes are not inherited. The idea involved is comparatively old. Vilmorin's promulgation of his "isolation principle" in plant breeding in the middle of the nineteenth century might be called its starting point. Vilmorin used the average character of a plant's progeny as the index of that particular plant's breeding capacity. This is the genotype conception, pure and simple. Since that time all plant breeding by selection which has been at all profitable has been done in this way, although the theoretical interpretation of the results obtained was unknown. This was given by Johannsen through his work upon barley and beans.

Since then corroborative results have been obtained by Jennings (1908, 1910) on Paramaecium, Hanel (1907) upon Hydra, Pearl (1909, 1911) upon fowls, Barber (1907) upon yeasts, Woltereck (1909) upon Daphnia, Jensen (1907) upon bacteria, East (1910a) upon potatoes, Love (1910) upon peas, and Shull (1911) and East (1911) upon maize. And no one to my knowledge has made a successful attack upon the position taken. It is true that attacks have been made by Pearson (1910) and Harris (1911), but their main argument is that the genotype theory is wrong, because it antagonizes the utterly erroneous biometric idea that heredity is measured only by the correlation between parents and progeny in somatic characters.

To be sure a caveat has been filed by Castle ("Heredity", New York, 1911) to the effect that unit characters so called can sometimes be modified by selection. This is no real criticism of the genotype conception of heredity, however, for Castle firmly believes in the generality of Mendelism and the general noninheritance of somatic modifications. It must simply be understood that, like

most chemical compounds, characters are generally stable under ordinary conditions, but also like chemical compounds they may sometimes be modified. This modification then becomes a new character or is the old character in a slightly different form, depending on the point of view.

The second part of the proposition rests upon the law of segregation and recombination of gametic factors, which is the essence of Mendelism. Every day the generality of this law becomes more probable. Leaving out of consideration experiments on apogamous and parthenogenetic species almost every paper published since 1900 dealing with crosses between varieties fertile inter se in which qualitative differences have been studied has shown that factors representing these characters segregate in the germ cells of the hybrid and recombine in the next generation. The few exceptions have been papers dealing with characters evidently quantitative, treated from a biometrical standpoint and not proving or disproving anything.

Recently there have also been investigations (Emerson, 1910; East, 1910, 1911; East and Hayes, 1911; Lang, 1911, Tammes, 1911) showing that size or quantitative characters also segregate. Of course all selection experiments on cross-fertilized species using Vilmorin's isolation principle and the investigations just cited in support of Johannsen have really proved segregation and recombination of size characters, else strains differing in such characters could not be isolated from complex hybrids. The senior writer (1910), however, has shown how such segregation can be given a strict Mendelian interpretation by postulating absence of dominance and multiplicity of determinants affecting the same general characters. The experimental basis upon which it rests is the investigations of Nillson-Ehle (1909) upon oats and wheat and his own upon maize.

It is possible that there are many apparent exceptions to the law of segregation; it is even possible that practically there are real exceptions, but these exceptions are likely to be in the nature of changed conditions which modify the action of Mendel's law through new sets of conditions. Our meaning is shown by parallels in the domain of physics and chemistry, where certain laws act perfectly only under ideal conditions which are very often not fulfilled in nature. For example, De Vries (1907) states that Burbank's and Janczewski's bramble hybrids have bred true. Without any data upon which to base a critical judgment one does not know what to say, but taking the statement at full value, any number of conditions may cause this hybrid constancy without invalidating the law of segregation. There may be apogamy, all zygotes may not develop,

selective fertilization may occur, or the action of the law may be opposed or suspended by other conditions of which we know nothing.

Personally we consider the genotype conception not as a theory but as a fact. Considering it as a fact, how does it aid the interpretation of the results obtained by inbreeding and by crossing inbred types of maize? Maize as a cross-fertilized species of great variability is in a constant state of hybridization. It is a collection of complex hybrids. Its usual mode of pollination through the agency of the wind keeps up this state of hybridization. Inbreeding, however, tends to produce homozygous types. As already shown, if one assumes equal fertility for all plants and that each plant lives and produces offspring in the n^{th} generation there is a ratio $2^n - 1$ pure dominants, 2 heterozygotes and $2^n - 1$ pure recessives for each allelomorphic pair.

This theoretical state of affairs may not occur for other reasons (as unpaired chromosomes) and the large number of allelomorphic pairs in a complex hybrid may prolong the time required for isolation of strains that are completely homozygous, but final isolation of strains completely homozygous is the goal toward which inbreeding tends. These completely homozygous strains are Johannsen's homozygous genotypes. Perhaps no one has ever isolated a real homozygous genotype, but strains homozygous for many characters are constantly being separated. This, indeed, is the sole function of selection.

Weismann assigned two purposes to the gametic fusion termed sexual reproduction; one being to mingle the hereditary characters carried by the two germ cells, the other to stimulate development of the zygote. This general statement was so obviously a fact that biologists were unanimous in its acceptance and two distinct lines of investigation have developed from it. Research concerning transmission phenomena has been almost divorced from the study of the physiology of development in its intimate connection with sexual reproduction. This separation, in view of the subject of this bulletin, seems unnecessary and unwise, for it may permit only a partial and distorted view of the results of reproduction. At any rate the data given here are of interest from both view points, since they deal with a purely physiological result brought about by a strictly morphological transmission phenomenon.

The hypotheses in regard to the way by which the act of fertilization initiates development are numerous, but since they are entirely speculative it is not necessary to discuss them here. The only conclusion that seems justified is that they are not immediately psychological or vitalistic in nature. Loeb's remarkable researches prove this. But whatever may be the explanation of the means by which the process

is carried out, the statement can be made unreservedly that the heterozygous condition carries with it the function of increasing this stimulus to development. It may be mechanical, chemical, or electrical. One can say that greater developmental energy is evolved when the mate to an allelomorphic pair is lacking than when both are present in the zygote. In other words, developmental stimulus is less when like genes are received from both parents. But it is clearly recognized that this is a statement and not an explanation. The explanation is awaited.

The developmental stimulus is to a certain degree cumulative. In other words, the expression "the greater the degree of heterozygous condition the greater is the vigor of the resulting plant" roughly expresses the facts. This does not mean that the possession of certain allelomorphic pairs in a heterozygous condition is not more necessary than others of normal development. Castle and Little (1910), for example, have shown the probability that zygotes which are potentially homozygous yellow mice are formed but do not develop. Baur (1909) has shown that homozygous recessives of pelargoniums that lack the necessary mechanism for chlorophyll formation are formed but can live only a few days. Of course in the latter case there is actual absence of a physiological mechanism that is absolutely essential to development. Whether the condition is similar in the yellow mice is unknown. It is quite possible that lack of normal or presence of abnormal factors will account for many cases of improper development, but these facts must not be confused with the phenomenon under consideration. What we are concerned with here is that developmental stimulus due to heterozygosity increases roughly with the number of heterozygous allelomorphic pairs, even though some of these pairs may produce a much greater stimulus than others.

Inbreeding, then, tends to isolate homozygous strains which lack the physiological vigor due to heterozygosity. Decrease in vigor due to inbreeding lessens with decrease in heterozygosity and vanishes with the isolation of a completely homozygous strain. Moreover, these homozygous strains can be quite different from each other in natural inherent vigor. From a single strain of Leaming dent maize one isolated type is a good profitable corn after four generations of inbreeding, having yielded at the rate of 80 bushels per acre in 1910; another type is partially sterile and can hardly develop to maturity after five generations of inbreeding, and yielded in 1910 only 9.5 bushels per acre. Thus we see the true explanation of the apparent degeneration that so many observers have attributed to inbreeding per se.

When species that are naturally close fertilized produce variations that are weak and degenerate, they perish in the natural struggle for existence or are not allowed to propagate by man. Since only the experimental breeder sees the origin of degenerate strains of close-fertilized species (as we have done in the genus Nicotiana), biologists have left them out of their consideration and have concluded that some exception to the natural laws of physiology has been made in their favor so that they could stand the inbreeding for which they are naturally fitted. Nothing could be further from the facts. Species which through their flower structure must be self-fertilized produce as many degenerate strains as any species. They are produced, but they do not survive; they are lost and forgotten. Species which through their flower structure are naturally cross-fertilized, on the other hand, produce strains poor in natural vigor, degenerate strains, and they are kept from sight. They survive the scythe of natural selection; they are selected for propagation by man because they are crossed with other strains and are vigorous through heterozygosity. Inbreeding tears aside their mask. They must then stand or fall on their own merits. Those strains with a high amount of inherent natural vigor, due to gametic constitution, lose the added vigor due to a heterozygous condition, but are still good strains, ready to stand up forever under constant inbreeding. The poor strains that have had the help of hybridization with good strains, combined with the added vigor due to heterozygosity, are stripped of all pretense, shown in all their weakness, and inbreeding is given as the cause for their degeneracy. At least inbreeding has until recently been given as the cause, but it is hoped that the newer interpretation will now be accepted as logically interpreting all the facts.

Although the increased power of growth of hybrids and the decreased vigor attending inbreeding have not been recognized as the same phenomenon until the work of Shull and the senior writer, nevertheless there has been a so-called interpretation of the increased vigor of hybrids current among plant physiologists. It is the theory of rejuvenescence or renewal of youth in the protoplasm. Continued self-fertilization is thought to be comparable to vegetative reproduction and continued vegetative reproduction is supposed to bring about a senile condition in the protoplasm. This theory was borrowed from zoology, having long since been proposed by Bütschli to account for conjugation in protozoa. It can not be considered a theory that helps in interpreting the vigor of hybrids, for it tells us nothing. Moreover, it may be based upon wrong premises. It is not at all certain that conjugation is an absolutely necessary phenomenon. Woodruff (1911) has demonstrated that protozoa can be kept in

healthy condition without conjugation for at least 2,300 generations. Jennings has been unable to make certain genotypes of Paramaecium conjugate. Nuclear fusions sometimes occur in some of the ascomycetes and basidiomycetes, but in general these fungi reproduce asexually and possibly have produced hundreds of species in this manner. In the higher plants there are many species in which either no seed is produced or sexual propagation is seldom resorted to, and yet they seem to be in no danger of degeneration. Among them may be mentioned the banana, hop, strawberry, sugar cane, and many of the grasses. There are also certain apogamous genera, such as Taraxacum and Hieracium, that are exceedingly vigorous. From these facts it is reasonably conclusive that sexual reproduction may be a benefit, but is not a necessity.

Keeble and Pellew (1910) have recently suggested that "the greater height and vigor which the F_1 generation of hybrids commonly exhibit may be due to the meeting in the zygote of dominant growth factors of more than one allelomorphic pair, one (or more) provided by the gametes of one parent, the other (or others) by the gametes of the other parent." We do not believe this theory is correct. The "tallness" and "dwarfness" in peas which Keeble was investigating is a phenomenon apparently quite different from the ordinary transmissible size differences among plant varieties. Dwarf varieties exist among many cultivated plants, and in many known cases dwarfness is recessive to tallness. It acts as a monohybrid or possibly a dihybrid in inheritance, and tallness is fully dominant. Varietal size differences generally show no dominance, however, and are caused by several factors. Transmissible size differences are undoubtedly caused by certain gametic combinations (East, 1911), but this has nothing to do with the increase of vigor which we are discussing. The latter is too universal a phenomenon among crosses to have any such explanation. Furthermore, such interpretation would not fitly explain the fact that all maize varieties lose vigor when inbred.

EXTENSION OF THE CONCLUSIONS TO THE ANIMAL KINGDOM.

Can the conclusions in regard to heterozygosis be extended to animals? The answer is affirmative as far as an interpretation of the known facts is concerned. No experimental attack from the standpoint taken in this paper has been made, but the older work furnishes many data that readily fit this view. As a matter of fact, however, it is questionable whether it is necessary to make formal proof in the matter. Sexual reproduction has undoubtedly arisen several times in the vegetable kingdom and at least once independently in the animal kingdom. Why or how it arose, one need not

inquire; having arisen, the purposes served are essentially the same if the similarity of the methods is an argument. The duplex nature of organisms, the halving of the chromatin and the production of simplex cells at the maturation of the sex cells, the fusion of two simplex cells as the starting point of a new organism, the general result of this fusion in the matter of development, and the transmission of heritable characters, are so similar in their main points that it would be quite wonderful if the process both in plants and animals did not now fulfill like requirements.

Since our conclusions are based upon the generality of Mendelism, which has been rendered highly probable by the multiplicity of zoological researches, it seems only necessary to show that heterozygosis in animals does cause (or accompany) an increase in vigor. It is easier to do this than to attack the still widespread belief that inbreeding is injurious per se. We have seen fertile crosses between different varieties of cattle, of swine, of sheep, and of domestic birds that were more vigorous than either parent. There are several swine raisers in the Middle West who make a practice of selling only first-generation crosses on account of their size. A number of very vigorous sterile hybrids of both domestic and wild animals might also be cited, but with these crosses a complication is encountered. In plants we found that the presence or absence of normal sexual organs made little if any difference in the amount of vigor induced by heterozygosis. In animals the case is undoubtedly different. From their very mode of development—animals being closed forms and plants open forms—internal secretions play a great rôle. And it is a matter of common knowledge that castration, in vertebrates at least, causes an extraordinary development of the body. In the human race this development is especially noticeable in the femur bones, so that Havelock Ellis states that the eunuchs of Cairo can be readily picked out of a crowd by their great stature. It is obvious, therefore, that there are two causes of vigorous somatic development, elimination of sexual organs and heterozygosis. In sterile hybrids, therefore, one can not say how much of the induced stimulation is due to each cause, but in fertile crosses there is no question about its source.

It is much more difficult to argue against the supposed injurious effects of inbreeding. Abhorrence of incest, which probably had a religious origin among our ancestors, is so difficult to eradicate from our minds that judgment is made before the facts are heard. This belief is not universal in the human race if Westermarck, the greatest authority on the history of marriage, is to be trusted, but the retort is made that the races that approve incestuous unions are low in intelligence. The answer does not prove anything, however, as low races with both beliefs are found, and, furthermore, as disapproval of inces-

tuous relations is both religious and esthetic, it would only be expected in races of some intelligence. Nor is the answer germane, for it is not shown that incestuous tribes are less well developed physically than related tribes with different customs, which is the real matter under examination.

But let us confine the discussion to the lower animals. If this is done there are two things to consider, the closeness of matings and their result. The statement is often made that self-fertilization in plants is a much closer sexual relationship than can obtain in bisexual animals. With a germ-to-germ transmission conception of heredity it is doubtful if this is true. After a wide cross, a self-fertilized plant of the F_1 generation produces markedly different progeny, due to recombinations of gametic factors. After continuous self-fertilization for many generations, the gametic factors tend to become homozygous and their matings are close in relationship. Thus it is perfectly clear that it is not kinship of the two organisms furnishing the sex cells that determines the closeness of the mating, but the similarity of the constitution of the cells themselves. There is no a priori reason, therefore, why bisexual animals may not be bred as thoroughly in-and-in as plants.

On this account the statement must be made very emphatic that investigations such as studies of cousin marriages in the human race amount to nothing. A cousin marriage may be a wide cross, it may be very narrow.

There is a possibility that has not been mentioned, however, that may prove to be an essential difference between the reproduction of bisexual animals and hermaphroditic plants. There is no question but that sex in the higher animals is essentially Mendelian in its behavior. There is no necessity of tying its interpretation to the chromosomes or to the accessory chromosome in particular. Castle's (1909) simple explanation that the female is gametically [1] the male plus a theoretical X factor has interpretated so many facts that its correctness—possibly somewhat modified—is highly probable. Under this interpretation one sex is always heterozygous. No similar explanation has been advanced to account for hermaphroditism. Possibly the same thing accounts for the differentiation into microgamete and macrogamete in plants, although not accompanied there by somatic changes. Since we are ignorant of the facts in plants, we can not say that sex furnishes a real reason for believing bisexual animal matings

[1] Note the words "gametically the male." This is not at all the same thing as saying *the male plus* something else. The X may produce many important changes during ontogeny.

There are two classes of facts; in one the male is homozygous, having no X factors, while the female has one. In the other the male is heterozygous, having one X factor, while the female is homozygous, with two X factors. The human race probably belongs to the second type.

less incestous than plants. The facts are simply given for what they are worth.

We are now ready to take up the actual effect of inbreeding in animals. In the statements of Darwin's correspondents we find throughout the tendency to mix esthetic feelings and facts. But here and there an independent observer maintained that breeding good stocks in-and-in had no evil effect. Undoubtedly there is sometimes a slight loss in vigor (we should say vegetative vigor as we have done in plants, because constitutional vigor is not lost), but there is no degeneration. On the other hand, there is segregation toward homozygous strains, and these strains differ in constitutional vigor. The greatest breeds of horses, cattle, swine, and sheep have been developed by in-and-in breeding. Breeders have worked for homozygous strains, for they desired strains that bred true. Inbreeding has been accused of producing everything undesirable in many of these strains, but the accusations are extremely illogical. Consider one or two examples. The race horse has undoubtedly been inbred more than the draft horse. Did inbreeding produce the nervousness and delicate constitution of the former? Certainly not. It is absolutely essential that the race horse be nervous. It has been thus selected for generations. Again, the delicate constitution of the Boston terrier or even the toy terrier is pointed out as the effect of inbreeding. We doubt very much if there has been any more inbreeding in the case of the Boston terrier than with the St. Bernard, but the selective ideals have been quite different.

The necessity for heterozygosis may be very different in various species of animals. In some the stimulus to zygotic development may be insufficient when like germ cells conjugate; in others, it may produce normal development. Weismann has made much of the fact that hermaphroditic animals are always cross-fertilized at times. It may be necessary in these species or it may be coincidence. Possibly hermaphroditic species will be found that are always self-fertilized yet retain their vigor even as in plants. At any rate Weismann's arguments seem to have little force, considering the widespread prevalence of parthenogenesis in the animal kingdom. It seems reasonable to conclude that in animals as in plants cross-fertilization may be advantageous but is not a necessity.

The actual experiments of Crampe (1883), Ritzema Bos (1894), and Von Guaita (1898) on mammals, of Fabre-Domengue (1898) on birds, and of Castle et al (1906) on the fly *Drosophila ampelophila* Low may all be interpreted in this way. Fertility was decreased in some strains. Those strains needed the stimulus due to a certain amount of heterozygosis for their proper development. Other strains

were perfectly fertile in spite of inbreeding. Sometimes combinations of hereditary characters resulted in relatively weak strains; other combinations of characters gave strong strains. In no case was there absolute and universal degeneration due directly to inbreeding.

As a final example of the simple way in which these experiments on animals fit the heterozygosis theory, we will take a case that puzzled Weismann (1904). Nathusius allowed the progeny of a Yorkshire sow to inbreed for three generations. Weismann says: "The result was unfavorable, for the young were weakly in constitution and were not prolific. One of the last female animals, for instance, when paired with its own uncle, *known to be fertile with sows of a different breed*, produced a litter of 6 and a second litter of 5 weakly piglings; but when Nathusius paired the same sow with a boar of a small black breed, which boar had begotten 7 to 9 young when paired with sows of his own breed (the black breed evidently near homozygous through close breeding), the sow of the large Yorkshire breed produced in the first litter 21 and in the second 18 piglings."

VALUE OF HETEROZYGOSIS IN EVOLUTION.

Before undertaking to discuss the part that heterozygosis may have played in evolution, emphasis must be laid upon one point of criticism directed against almost all speculative evolutionary philosophy. Unconsciously, perhaps, many of the conditions that are widespread among living forms have been spoken of as having been selected to continue their existence in nature because they are indispensable to the organism. This is certainly untrue. One has only to recall other epochs of geology to appreciate the fact. The huge reptiles of the Cretaceous period were long in developing their peculiar specializations, yet they were swept away. In a present-day post-mortem we can assign many reasons why they were eliminated from the organic world, but if their characters were so unfit for their environment, how did they come to be developed? It is said the environment changed and left them too specialized for adaptive response. This is plausible enough, but, nevertheless, possibly untrue.

Must we not be just as skeptical about the question of sexual differentiation? It has arisen several times; it has persisted. Having arisen, it undoubtedly has a function. Perhaps it was necessary; perhaps it was a fundamental blunder, as was once humorously stated. Speculation is, of course, futile. We merely wish to point out that in discussing a function intimately connected with sexual reproduction it is absolutely unnecessary to suppose that sex fulfills

a protoplasmic necessity or demand.[1] We do not say that the belief is untrue, but that it is not known to be true and therefore should not be treated as a fact.

In other words, electric drills and hammers are very useful in building a bridge, but good bridges have been built without them. Sexual reproduction serves a purpose, but several of the most vigorous genera of our higher plants have given it up. It is evidently unnecessary to them. We must cast a vote, therefore, against the belief in the rejuvenescence theory of sexual reproduction. Furthermore, we believe that any hypothesis in which an endeavor is made to twist the phenomena attending sexual reproduction into requisites indispensable to the evolution of all species possessing it is without basis. All one can do is to suggest how it may have been beneficial at times to some species.

Transmissible variations are produced in great numbers by apogamous genera such as Taraxacum and Hieracium, so that sexual reproduction is not the cause of variation. Johannsen's (1906) and many other pedigree-culture studies have shown that it presumably never increases variation. But it does permit recombination of the gametic factors of the parents, and this has no doubt been of great service in evolution. Galton and Quetelet (Weismann, 1904) have argued that the intercrossing thus allowed is a means of keeping the species constant, but even with the old idea of blended inheritance this seems to us to be an exaggeration. Greatest constancy in the actual descendants, if new heritable variations are disregarded, would come from asexual reproduction. If the species group is considered as a whole and comparatively free from competition, a great amount of intercrossing—as in a naturally cross-fertilized strain—would help toward a general fixation of type, even though it did not contribute toward the production of homozygous factors; but if a rigid competition is allowed, new and better combinations of characters would replace the old. Perhaps this matter may be made clearer by an illustration drawn from our maize studies. Height is a complex due to many contributing factors. Some of them are probably correlated in inheritance, but a sufficient number are transmitted independently to give the

[1] Vitalistic interpretations of the origin of characters, though largely confessions of ignorance of ultimate causes, deserve consideration for calling attention to that fact; yet one must admit that if everything is accounted for by some "perfecting principle" this creative force has made many trials and errors. Of course things do not just happen. The chemist sees certain series of compounds give similar reactions under like conditions, while other series give other reactions under those conditions. More complex chemicals under the general term protoplasm probably act in the same manner and produce variations through their reactions. Some of these variations are widespread—that is, they are general reactions; others are less general—that is, they are specific reactions. Personally this analogy helps in the conception of certain orthogenetic phenomena, but the conception leads back to the same blank wall of ignorance. The vitalist and the believer in mechanico-chemical theories reach the same point, but the latter is pleased if he is able to reduce a series of facts to the shorthand of a formula; the former is worried because knowledge stops at the most interesting place.

example validity. There is no dominance, and when two individuals differing in stature are crossed there is a blend in the first hybrid generation. There is a real segregation, however, resulting in an increased variability in the F_2 generation. In the F_1 generation there is a normal frequency deviation due to noninherited fluctuations. In the F_2 generation there is a similar curve, but with greater variability, due to fluctuating variability plus the variability due to the recombination of gametic factors. This condition of affairs tends toward the maintenance of a general mean in height, but this mean is false. It is false because the modal class which Galton and Quetelet took to be the type toward which the species is tending actually contains more heterozygous individuals and individuals heterozygous for more factors than any other. An individual selected from this class is less likely to breed true than one selected from the extremes. Cross-fertilization, therefore, may tend toward the production of a mean that gives falsely an appearance of fixity of type.

This preliminary discussion has necessarily been rather long in order to have a basis for considering the part that heterozygosis may have played in evolution. We shall confine ourselves to the higher plants, although we think a portion of the statements made are equally true when applied to animals. It can hardly be doubted that heterozygosis did aid in the development of the mechanisms whereby flowers are cross-fertilized. Variations must have appeared that favored cross-fertilization. These plants producing a cross-fertilized progeny would have had more vigor than the self-fertilized relatives. The crossing mechanism could then have become homozygous and fixed, while the advantage due to cross-fertilization continued. But was this new mechanism an advantage? It must have been often an advantage to the species as a whole. In competition with other species, the general vigor of those which were cross-fertilized would aid in their survival. But the mechanism may not have been useful in evolving real vigor in the species, because of the survival of weak strains in combination. In self-fertilized species, new characters that weakened the individual would have been immediately eliminated. Only strains that stood by themselves, that survived on their own merits, would have been retained. On the other hand, weak genotypes in cross-fertilized species were retained through the vigor that they exhibited when crossed with other genotypes. The result is, therefore, that self-fertilized strains that have survived competition are inherently stronger than cross-fertilized strains. On this account weak genotypes may often be isolated from a cross-fertilized species that as a whole is strong and hardy.

VALUE OF HETEROZYGOSIS IN PLANT BREEDING.

First-generation hybrids of many economic plants give a yield sufficiently greater than pure strains to pay for their production and leave a profit. This is true only of crops where crossing is easy and where profit is made from accelerated and increased cell division or number of fruits. In general, it is not true where the selling price is greatly increased by the possession of some special quality. As Collins has remarked, value may at times accrue also from the fact that a plant breeder who has found a great increase in yield from growing the first hybrid generation of a particular cross may keep the parents a secret and maintain a justly remunerative business by selling hybridized seed or seedlings. A few suggestions as to the crops to which this method may be applied are given below.

MAIZE.

Maize is our most important field crop, and an increase of one bushel per acre to the average yield would add many millions of dollars annually to the nation's resources. The methods now in general use for its improvement all follow Vilmorin's isolation principle. Progeny-row tests are grown from individual ears. This means that good strains are isolated, but it also means that the longer selection is carried on the nearer is a homozygous condition approached. Thus the increased stimulus due to heterozygosis is lost. Since from both Shull's tests and our own, strains made almost homozygous by artificial inbreeding have yielded as high as 250 per cent increase over the average of the parents, this stimulus is not to be lightly disregarded. Of course these tests were made with strains so nearly homozygous that they gave very low yields. But we have obtained yields of ear corn very much higher than are ever given on land of like fertility by commercial types. Shull (1909) has therefore suggested that near-homozygous strains be produced by self-fertilization, the best combination determined by experiment, and hybridized seed of this combination sold. This procedure is undoubtedly the best in theory, because the greatest degree of heterozygosis is thereby obtained. Perhaps it can be made practical, but we are afraid very few commercial men would undertake it.

As a method whose practicability outweighs its theoretical disadvantage, the senior writer (East, 1909) has suggested that combinations of commercial varieties be made, testing them until the most profitable combination is found. Since maize is monœcious, this method can be used on a large scale at a small cost. It is only necessary to take two varieties, *A* and *B*, plant them in alternate rows, and detassel all of the plants of one variety. The seed gathered

from this detasseled variety is all crossed seed and will give, in general, a greater yield than the average of the two parents. Crossed seed can be produced in this manner at an additional cost over natural seed of not over 9 cents per bushel. If it averages two bushels per acre increase in yield, the producer can sell it at one dollar advance over natural seed and still allow the buyer a good profit. The method is given in greater detail in another paper (Hayes and East, 1911).

This plan we thought original, but Collins (1910) has shown that it is comparatively old. It has been suggested time and again without gaining a foothold in agricultural practice. Let us hope that the time is now ripe for a scientific method to be understood, appreciated, and used.

It is fortunate that we have at hand data from many agriculturists showing the value of using first-generation hybrids in maize. They are very convincing. We will not discuss them in detail, but refer the reader to Collins's paper (1910). We may say, however, that the following researches have shown that a commercial use of the method is possible: Beal at the Michigan Experiment Station in 1880, Ingersoll at the Indiana Experiment Station in 1881, Sanborn at the Maine Experiment Station in 1889, Morrow and Gardner at the Illinois Experiment Station in 1892, Shull of the Carnegie Institution Station for Experimental Evolution in 1909, East at the Connecticut Experiment Station in 1909, Collins and his assistants of the United States Department of Agriculture in 1910, Hayes and East at the Connecticut Experiment Station in 1911, and Hartley and his assistants of the United States Department of Agriculture in 1912.

TRUCK CROPS.

In some truck and garden crops, such as beans and peas, the difficulty of making artificial crosses absolutely precludes a commercial use of the stimulus due to heterozygosis. Other crops, such as pumpkins and squashes, are too plentiful and cheap to be worth the trouble. Besides, these crops are so often crossed naturally that they are always more or less heterozygous. On the other hand, there are garden crops that are in demand at all seasons of the year and are grown under glass during the winter with profit. Some of them are easily crossed and will pay for their crossing. As examples, tomatoes and eggplants may be cited. Both are easily crossed and are worth crossing. We grew a cross between Golden Queen and Sutton's Best of All tomatoes in 1909. It outyielded both parents. Further, we are informed that several unpublished experiments at the New York Experiment Station by Wellington have shown that crossed seed is worth its production.

Eggplants have another advantage that should be mentioned. Varieties exist whose fruits are so large that the buyer does not care for them, the seller makes no profit, and the plant produces a very limited number. Other varieties have very small fruit. Now fruit size is intermediate in the first hybrid generation, while the number produced is increased and the time of ripening advanced.

PLANTS REPRODUCED ASEXUALLY.

The one type of plants where heterozygosis has been utilized, though not purposely, is that class which is reproduced asexually by cuttings, grafts, etc. Potatoes and grapes are good examples. Commercial varieties are always hybrids, and the reason, we think, is because the hybrids yield so profusely. The cross is made and the best plant of the first generation is simply multiplied indefinitely by division. This method could be applied more generally to bush fruits, such as gooseberries, raspberries, blackberries, etc., and to the larger fruits, like apples, pears, and peaches.

FORESTRY.

There is one other class of economic plants where it seems possible to make a practical use of heterozygosis. We refer to trees used for lumber. Many plans for breeding forest trees have been suggested, yet we have never seen the use of first-generation hybrids suggested. This omission seems strange, for as early as 1855 (Darwin, "Animals and Plants," vol. 2, p. 107) M. Klotzsch crossed *Pinus sylvestris* and *nigricans*, *Quercus robur* and *pedunculata*, *Alnus glutinosa* and *incana*, *Ulmus campestris* and *effusa* and planted the crossed seeds and seeds of the pure parent species in the same place and at the same time. The result was that after eight years the hybrids averaged one-third taller than the parent trees. Further, the quick-growing hybrid walnuts produced by Luther Burbank undoubtedly owe that valuable quality to heterozygosis.

A large amount of experimental work will be necessary before definite recommendations can be made as to what species can be crossed, what result may be expected, and what extra cost must be allowed for the production of hybrid seed. It is perfectly evident that hybrid seed will be impossible in many cases, and even where hybrids can be produced comparatively few can be crossed at a small enough cost to make the scheme a commercial success. On the other hand, we have no doubt that with many good lumber trees crossing would be found easy and hybrid seed could be sold with a wide margin of profit both to producer and to forester.

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